THE EFFECTS PRODUCED ON PARENTS AND DESCENDANTS BY THE INJECTION OF CALF LENS MATERIAL DIRECTLY INTO THE RABBIT PARENT[1]

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INTRODUCTION

In a previous paper (IBSEN and BUSHNELL 1931) we presented the results obtained from the employment of two of the three methods used by GUYER in the production of eye defects in the offspring of treated pregnant rabbits. The two methods were: (1) the injection of hen blood-serum containing lens antibody into the blood system of the rabbit, and (2) the needling of the lens of the rabbit in order to enable it to produce its own antibody. Our results differed from those of GUYER in that we were unable to obtain any conclusive evidence that the treatment of the mothers had any effect on the offspring.

One objection to the above two methods is that there is little or no possibility of obtaining treated rabbits with a large amount of lens antibody. Even if the blood serum of the hen has a high precipitin titer, there will be a marked dilution after it is introduced into the blood system of the rabbit. When the lens is needled there is still less possibility of a high titer. The reason for this is that the amount of lens material involved is relatively small, and, besides, the blood does not have easy access to it, thereby tending to cause a reduction in the rate of absorption. These points are well emphasized by the fact that we were unable to demonstrate the presence of any antibody in the blood of needled animals.

The third method used by GUYER seemed to offer greater possibilities of success because of the apparently better opportunity for increased precipitin titer in the treated animals. One would expect that animals with a high lens antibody content would have less difficulty in affecting the eyes of the unborn young than would those with a low antibody content. The method consisted in the injection at definite intervals of lens material directly into the rabbit. Treating hens in this manner produced a high titer in at least some of the individuals, and it was reasonable to assume that similar results could be obtained with rabbits.

GUYER himself, however, did not use this method to any great extent (GUYER and SMITH 1924), and it was for this reason chiefly that we tried the other two methods first. Another reason was that he obtained only a

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small number of defective-eyed young from his eleven treated females. The latter were injected intravenously with a mixture containing the pulped lenses of young rabbits in normal salt solution. Each injection usually contained the equivalent of slightly more than one lens. The injections were made on the average about a week apart, and each rabbit received from four to thirty-two, the average being nineteen for the eight animals whose records are given in detail. Those that were given more than eleven injections, however, did not receive all of them a week apart; over a month intervened in one case and about three months in another.

GUYER's eleven treated females produced a total of seventy-one offspring. Seventeen of these died before their eyes opened, and were discarded. Two of the remaining fifty-four had defective eyes, and both came from the same female (No. 84). None of the treated females, so far as reported, were ever tested for lens antibody content, thus making it impossible to determine whether greater frequency of eye defects in the offspring was correlated with high precipitin titer in the mother.

EXPERIMENTAL RESULTS

In our direct injection (third method) experiments we did not follow GUYER's technique to the letter. In the first place we used calf lenses instead of those of the rabbit because the use of the former reduced the cost of the experiments materially, and also because it seems to be an established fact, and our experiences bear this out, that there is no appreciable difference in serological effect between the lenses of cattle and rabbits. Calf eyes, to any number, were liberally supplied us free of cost by the HYGRADE PACKING COMPANY of Topeka. This company is now owned by JOHN MORRELL AND COMPANY. Our technique differed in another respect from GUYER's in that the injections of lens material were made either subcutaneously or intraperitoneally, but never intravenously.

Our first three experiments were attended by one misfortune or another. Only the fourth was satisfactory. Almost every treated female in the first three experiments died before any offspring were produced, but many of them lived long enough to enable us to make a titration of the precipitin content of their blood.

First Calf Lens Experiment

The rabbits used in this experiment were from our colony, and some were descendants of the needled animals used in our previous experiments. There were six males and eleven females.

The calf eyes that were sent us were carefully washed in mercuric chloride, opened as aseptically as possible, and the lenses washed several times in sterile salt solution. A small amount of saline was added when the lenses

were pulped. All of the lenses in this and the succeeding experiments came from young animals as was evidenced by the fact that no hard cores were found in any of them. The average weight of the 150 used in the first experiment was 1.28 grams, or about four times as much as those of young rabbits.

The lenses were weighed before being pulped, and their combined volume with the saline solution was then measured. This made it possible to determine the weight of the lens material per cc of the lens suspension. In this first experiment each animal received 1 gram of lens per 2500 grams of body weight. The average initial weight of the seventeen treated animals was 2,442 grams. Five intraperitoneal injections were made at seven-day intervals, the first being given March 7, 1930. The fifth injection was made April 4, and ten days thereafter (April 14) 5 cc of blood were drawn from the ear of each of the seventeen treated animals and also from five untreated males.

Since calf lenses were used in producing the antibody, it was thought advisable to use both calf and rabbit lenses as antigens in making the precipitin titrations. The method of titration was similar to that described in our first paper (1931). The lens dilutions were not the same for all of the four experiments. In this experiment they were 1:80, 1:160, 1:320, 1:640, 1:1,280, 1:2,560, 1:5,120 and 1:10,240. By using both calf and rabbit lenses as antigens it should be possible to determine indirectly the degree of resemblance between the precipitins produced by the lenses of the two species. If the serum of the treated rabbits caused precipitation at approximately the same dilutions of the two antigens, it should be a good indication of serological similarity.

Table 1 furnishes a comparison of the two antigens. If an analysis be made of the data it will be found that seven animals gave the same titer with the two antigens, seven gave a higher titer with calf lens, and three with rabbit lens. There is evidence of a slight increase of titer with the calf antigen, but it is not significant statistically. Under the circumstances one is justified in assuming that by the use of calf lenses one has approximately the same opportunity of producing eye defects in the offspring of treated rabbits as by the use of rabbit lenses.

All of the treated rabbits gave indications of having some lens antibody, although a few caused precipitation at only the lowest dilution (1:80). On the other hand, the serum of the five untreated males which were used as controls gave no reaction at the 1:80 dilution when either kind of lens was used as antigen.

No live offspring were produced by any of the treated animals of the first calf lens experiment. This was mostly due to the fact that eight of the eleven females died of coccidiosis during the spring of 1930. The cause of

death of the ninth was supposedly a wire found in the stomach. The other two females (361A.2 and 367A.3) were mated on April 20, 1930 to 367A.1, a treated male (titer, 1:5, 120). About fifteen days after copulation it was determined by palpation that both were pregnant, but nevertheless no offspring were born. Some of the untreated females were behaving in a similar manner at this time, which prevents us from making the assumption that the fetal resorption was caused by the treatment the females

TABLE 1

Precipitin titers on the same animals, using both calf and rabbit lenses as antigens. The serum to be tested was drawn ten days after the last (fifth) injection.

PEDIGREE NUMBER OF RABBIT	TITER, USING RABBIT LENS AS ANTIGEN	TITER, USING CALF LENS AS ANTIGEN
♂358A.5	1:80	1:640
♂360A.1		1:640
♀367A.7		1:160
♂355A.5	1:160	1:160
♀367A.6	1:320	1:320
♂363A.1	1:1,280	1:10,240
♀355A.6		1:1,280
♀354A.6	1:2,560	1:5,120
♀363A.4		1:5,120
♀367A.3		1:1,280
♀367A.8		1:5,120
♂362A.1	1:5,120	1:5,120
♂367A.1		1:5,120
♀354A.4		1:5,120
♀356A.5		1:5,120
♀361A.2		1:1,280
♀357A.1	1:10,240	1:5,120

received. Both were again bred on May 19, 1930, ♀ 361A.2 to ♂362A.1 (titer, 1:5, 120), and ♀367A.3 to ♂367A.1. Fetal resorption occurred a second time in the case of ♀361A.2, while ♀367A.3 gave birth to one offspring, found dead on the date of birth, June 21, 1930. Both females were then carried over the summer and used in the second calf lens experiment.

The six treated males remained in apparently good health for a number of months. It was decided to make use of them in determining the length of time that the antibody remained in the blood. On June 9, 1930, sixty-six days after the fifth and last injection, blood was drawn and the serum

tested with rabbit lens as the antigen. Four of the males, 355A.5, 360A.1, 362A.1 and 363A.1, gave no reactions in lens dilutions of 1:20, while ♂358A.5 (whose previous titer was 1:80) gave a precipitation in a dilution of 1:20 and ♂367A.1 (whose previous titer was 1:5,120) in one of 1:40. There is probably as much variation in the length of retention of antibodies as there is in their quantitative production. These data, together with some obtained in the fourth experiment, also indicate that there is no relationship between titer and length of retention. In other words, a large amount of antibody may be lost just as rapidly as a small amount.

Second calf lens experiment

This experiment was begun in the fall of 1930. Seven males and seventeen females were used. Five of the males were from the first experiment. The sixth male from the first experiment, 362A.1, had died during the summer, apparently as a result of the excessive heat. As previously stated, two of the females were from the first experiment. All of the twenty-four animals had been produced in our colony.

The experiment had a disastrous ending. One of the shipments of calf eyes was delivered to the wrong building and was therefore temporarily lost. When it was finally found the ice in which it had been packed was melted and the eyes had become warm. Although extra precautions were taken with it, contamination was not entirely eliminated, and as a result all of the rabbits (except ♂389A.3) became infected and eventually died. They were in such poor condition after the last (fifth) injection that no blood samples were taken for the purpose of making a determination of the antibody content. The injections had been made intraperitoneally.

The above details would have been unnecessary were it not for the fact that one of the males, 355A.5, developed an opaque lens in the left eye after the third injection. He was one of the five males brought over from the first experiment and had therefore received eight injections altogether. His titer (1:160) was comparatively low when he was tested in the first experiment. It is possible that the opacity was due to the action of the antibody in his blood, although the possibility seems somewhat remote when one considers that one of GUYER's rabbits received thirty-two injections, and his eight animals received an average of nineteen, without any visible effects being noticed in the eyes.

On the other hand, one cannot say with any degree of confidence that the opacity was due to heredity. Both GUYER and ourselves have found apparently inherited cases that do not manifest themselves until after the animals are several months of age. The male 355A.5, however, was almost a year old when the lens became opaque. The best that can be done under the circumstances is to mention the case and leave it unexplained.

Third calf lens experiment

Our supply of rabbits had become so low by this time that it became necessary to obtain most of those for the third experiment from a dealer. They were guaranteed to be healthy, but proved otherwise. There were six males and twelve females. Six of the latter died of coccidiosis before the series of five injections were completed.

The injections were made October 31 and November 8, 14, 21 and 28, 1930. All were given the same dosage each time, which was on the average slightly more than 1 gram of pure lens per dose. The 105 calf lenses furnished us had a mean weight of 1.31 grams. The injections were subcutaneous, the skin of the middle of the back of the animal being perforated.

Ten days after the last injection, which would be on December 8, 5 cc of blood were drawn from the six males and six females that were still alive, and the serum titrated against calf lens made up to the following dilutions, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1,024, 1:2,048, 1:4,096, 1:8,192 and 1:16,384. The male with the least antibody caused precipitation in a dilution of 1:1,024, while the others were classified as follows: 1:4,096, one; 1:8,192, three; 1:16,384, one. The females were approximately the same—1:256, one; 1:1,024, one; 1:8,192, two; 1:16,384, two.

Although the titer of six females was obtained, only two lived long enough to reproduce, the other four either dying of, or being killed because of, coccidiosis. Female 415A.1, with a titer of 1:8,192 on December 8, 1930, was mated January 12, 1931 to ♂389A.3, left over from the second experiment, and whose titer was not determined. The litter of five born February 11 was normal-eyed. The mother's blood was tested February 13 and it produced no precipitation in any of the lens dilutions. The chances are that there was not much antibody in the blood of the mother during pregnancy. The offspring when inbred produced two litters containing fourteen individuals, all normal-eyed.

The other female, 405A.1, with a titer of 1:256 on December 8, 1930, was bred on January 12, 1931 to ♂399A.2 whose titer was 1:4,096 on December 8. A normal-eyed litter of four, all females, was born February 13. They were disposed of without being bred. The serum of ♀405A.1 was also tested on February 13, and, like ♀415A. 1, gave no precipitin reaction.

Fourth calf lens experiment

The first three experiments were to a large extent failures, but nevertheless enough was learned from them so that better plans could be laid for the fourth experiment. We knew that by the direct injection method rabbits could be obtained having a high titer. It seemed to us that if the treated female had a large amount of antibody, there should be a greater

opportunity for some of this to penetrate the placenta, thereby getting into the circulation of the fetus and producing an effect on the lens.

Taking the above facts into consideration, the breeding of the females was arranged in such a manner that some would receive their first injection toward the end of pregnancy, others would receive two injections before parturition, still others would receive three, four or five. It was also planned to obtain a second litter from each female. To produce such litters the females would have to be bred after all the injections were completed. These second matings were to be made a varying number of days after the completion of the injections. The interval in some cases would be so long that little or no antibody would be left in the blood. If defective-eyed offspring were produced under these circumstances it would indicate that the antibody had produced a direct effect previously on the immature germ cells. All of the males used were to be injected at the same time as the females in order to facilitate possible changes in their gametes.

By making all the matings outlined above, it was hoped that if eye defects appeared in any of the offspring one could arrive at a conclusion concerning the most favorable conditions for producing them. Although a large number of offspring were obtained, all were normal-eyed, so far as could be determined by the naked eye. The details are given herewith.

Most of the rabbits used were obtained locally from several sources and fortunately all of the animals were healthy with one possible exception. The experiment was begun with six males and twenty-six females. One of the latter died shortly after parturition, after having received three lens injections. Her litter was born dead. After the above animals had received the first injection, three more females were added to the experiment. Thus the total number that lived long enough to have titers made of their blood serum was six males and twenty-eight females.

The first injection was made January 30, 1931, and the remaining four on February 6, 13, 20 and 27. They were made subcutaneously in the middle of the back with a twenty gauge hyperdermic needle, the same kind as had been used in the previous experiments. The usual amount injected was 1.5 cc, consisting of calf lens and some normal saline. The average weight of pure lens per injection for the five injections was 1.031 grams.

We decided to continue the weekly injections with one male and four females for five more weeks, thereby giving them ten altogether. The average weight here of pure lens per injection was 1.251 grams. The dates were March 6, 13, 20, 27 and April 3, 1931. Altogether 226 calf lenses were furnished us for the fourth experiment, and they had a mean weight of 1.42 grams.

On March 9, ten days after the last injection for those which received only five (or four in the case of three animals) and three days after the

sixth for those which received ten injections, blood was drawn from all of the rabbits and the serum titrated against various dilutions of calf lens. The results are shown in table 2. If one combines these results with those obtained in the first experiment (table 1), taking the data obtained when calf lens was used as antigen, and with the twelve animals of the third experiment, the total will be found to be sixty-three. The titer for one-third of these, or twenty-one animals, is between 1:5,000 and 1:10,000.

There may be some difference of opinion in regard to the justification of placing all twenty-one animals in the above class since different methods of dilution were used in the three experiments. Of the remaining forty-two rabbits, twenty-eight had titers that were less than 1:5,000, and fourteen had titers of 1:10,000 or more, the highest being 1:25,000.

TABLE 2

Precipitin titers of the rabbits in the fourth calf lens experiment, March 9, 1931.

CALF LENS DILUTIONS	MALES	FEMALES
1:10		426A.1, 435A.1
1:100	376A.2	421A.1,† 422A.1,†‡ 430A.1 439A.1, 446A.1,*‡ 448A.1*‡
1:1,000	387A.1,† 398A.1	419A.1, 420A.1,† 425A.1 427A.1, 431A.1
1:5,000	392A.1	418A.1,‡ 423A.1,† 433A.1 434A.1, 437A.1, 441A.1 447A.1*
1:10,000	376A.1	428A.1, 429A.1, 438A.1
1:15,000		416A.1, 417A.1
1:20,000		436A.1, 440A.1
1:25,000	393A.1	424A.1

* Received only the last four injections.

† Received first six of a total of ten injections.

‡ Either failed to reproduce, or had offspring that died shortly after birth.

In our previous paper we reported titers on eighteen hens that had received rabbit lens intraperitoneally. One-half of them had titers of approximately 1:5,000, while the remainder were considerably lower. Thus in the small number of hens examined two distinct groups were obtained. In the rabbits, on the other hand, where the numbers are larger, there is no indication of discontinuity in the classes. This apparent species difference is probably due entirely to chance deviations.

Diagram 1 is a representation in graphic form of the fourth experiment breeding results in relation to the time of the lens injections. Each female that reproduced is listed in the vertical column on the left. Seven day intervals, with the exact dates, are represented from left to right across the diagram. The solid vertical lines represent the dates of injection. By inspecting these it will be noted, for example, that ♀ 447A.1 did not receive the first injection on January 30, and that females 420A.1, 421A.1 and 423A.1 received ten injections, the last one on April 3.

The vertical dotted lines represent the dates on which blood was drawn for the purpose of titrating the serum. The figures on either side of these lines represent individual titers. Instead of giving the dilutions, as for example 1:15,000 for 416A.1, the "1:" is omitted in each case. The horizontal lines represent the period of pregnancy for the various females. At the left end of these lines is placed the pedigree number of the father of the litter that was produced at the end of that particular pregnancy, and at the right end of the line the number of individuals in the litter that lived long enough so their eyes could be examined. The mortality in the litters was not in any respect unusual. For that reason it was not considered of importance to give the particulars.

It has already been stated that, so far as could be determined by the naked eye, all the offspring of the treated females in the fourth experiment had eyes that appeared to be perfectly normal. Diagram 1 gives the salient details in regard to 170 of these offspring. The remainder, which are seven in number, will be discussed later.

On examination of the diagram it will be noted that seventeen offspring were born after the mothers had received only one injection, which was administered toward the end of the period of pregnancy. Fourteen offspring were born to mothers that had received the first two injections, twenty-eight to mothers that had received the first three, and sixteen to those that had received the first four injections during pregnancy (the litter born to ♀ 447A.1 is included with these). There were twenty-three offspring born within two weeks after the mothers had received the last of five injections. These offspring should have developed under uterine conditions especially favorable to the production of eye defects, if the antibody carried by the mothers had had no difficulty in penetrating the placentas, yet all were normal-eyed.

Twelve litters, containing fifty-six individuals, were born to females bred from seven to thirty-two days after receiving the fifth (and also the last) injection. The twelve females had all been mated to males who had received five lens injections at the same time as themselves. For both male and female parents, therefore, an opportunity had been afforded the antibody to act directly on the immature germ cells. In addition, for all twelve

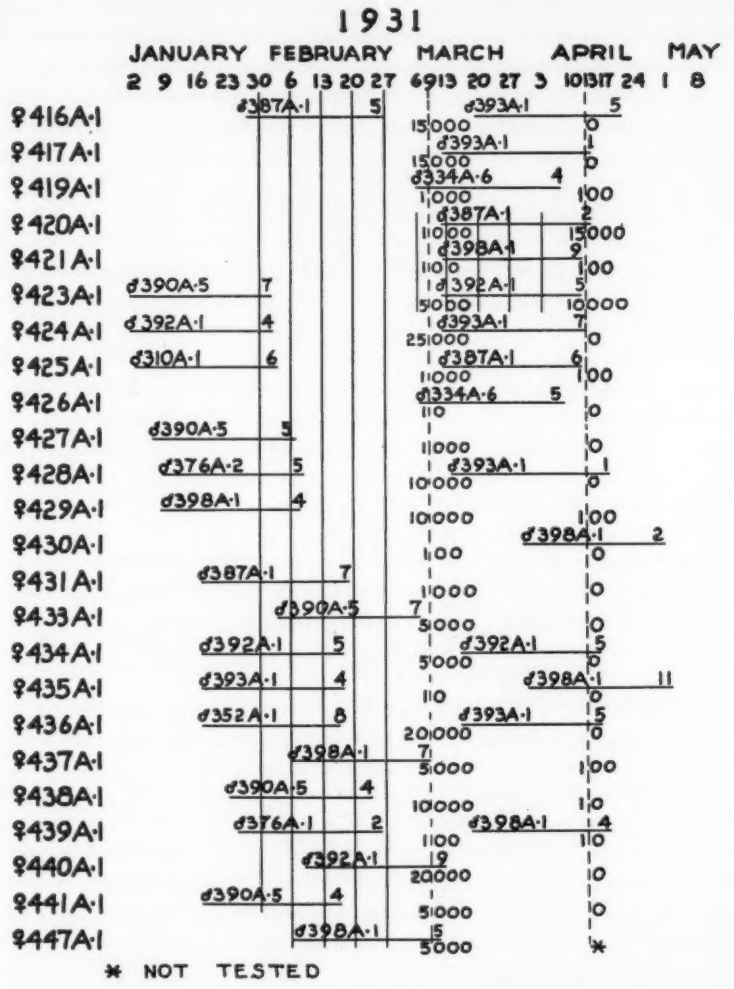

DIAGRAM 1.—Breeding results in relation to time of lens injections for the fourth calf lens experiment. The pedigree numbers of the treated females are listed at the left; dates are across the top. Solid vertical lines (five are long and five short) are dropped from dates of injections; dotted vertical lines from dates on which blood was drawn for the purpose of making antibody titrations. The numbers laid across the dotted lines indicate the titers of the females, and the short horizontal lines their periods of pregnancy. The male whose pedigree number is given at the left end of each gestation line is the one that was mated to the female. The number at the right end of each gestation line represents for that particular pregnancy the total number of young which lived at least fifteen days. All were normal-eyed.

litters, antibody was present in the mothers at least during the early stages of development.

Three other litters, containing sixteen individuals, developed under conditions that should have been very favorable for the production of eye defects. The mothers, ♀420A.1, ♀421A.1 and ♀423A.1, received ten injections, and the litters were *in utero* during the period covered by the last four of these. The fathers also had been injected at least five times and one of them, ♂387A.1, had received six injections before he was bred to ♀420A.1.

Most of the mothers were discarded shortly after the litters recorded in diagram 1 were old enough to be weaned. It would undoubtedly have helped to round out the experiment if, instead of being discarded, they had been retained over the summer and been bred again in the fall when, for at least most of them, there would not have been a measurable quantity of antibody left in the blood. If offspring with defective eyes had been produced under these circumstances it would lead one to believe that these were due to the direct action of the antibody on the gametes of the treated animals. The gametes that would give rise to offspring in the fall would have been relatively immature at the time the injections were made, and it seems possible that sensitivity to treatment is to some extent directly correlated with degree of immaturity.

Only one out of the twenty-four females recorded in diagram 1 was kept for any length of time and bred again. This animal, ♀421A.1, had received ten lens injections, the last one on April 3, and on August 12, 131 days later, she was bred to ♂387A.1, who also had been injected ten times. The litter of seven born September 12 contained only normal-eyed individuals. An attempt would have been made to produce more litters from this mating, but unfortunately ♂387A.1 died September 25, 1931, and the other four treated males of the fourth experiment had already been discarded. The above mentioned seven, added to the 170 listed in diagram 1, brings the total number of offspring in the fourth experiment to 177.

Female 421A.1 was subsequently bred to one of her sons, ♂451A.1, who was born before she had received any injections. Even if changes had taken place in her gametes they would not have been detected unless they behaved as dominant genes. Two litters containing nine offspring, all normal-eyed, were produced, but are not included in the totals.

One more reference should be made to diagram 1, and that is in connection with the titers obtained from blood drawn on April 13, 1931. The figures near the dotted line drawn through the above date represent the results. Wherever zero (0) appears it means that no precipitation occurred in any dilution, in other words, that there was no indication of antibody in the blood. This second titration was made forty-five days after the last

injection for those animals that were injected only five times. There were twenty such individuals, all females, and of these fourteen gave no reaction in any dilution, two caused a precipitation in a dilution of 1:10 and four in a dilution of 1:100.

Three females are included in diagram 1, which received ten injections and whose second titer was obtained from blood drawn the tenth day following the last injection. For two of these animals the second titer was higher than the first, while for the third there was no change.

The second titer was made on all the animals in the fourth experiment. Diagram 1 includes only those females that reproduced. Titers were also taken on all six males and on two females that failed to produce living offspring. Table 3 contains these additional data. Combining all the results (from diagram 1 and table 3), and taking into consideration those animals that received only five injections, it will be found that there were seventeen animals that carried no antibody forty-five days after the fifth injection, four that reacted to a dilution of 1:10, and five to a dilution of 1:100. As stated in the discussion of the results of the first calf lens experiment, there is no evidence here of any correlation between the amount of the antibody and the length of time it remains in the animal.

TABLE 3

Precipitin titers on April 13, 1931, of those animals of the fourth calf lens experiment not listed in diagram 1. (Two females, 446A.1 and 448A.1 were not tested on the above date and therefore are not included in the table.)

CALF LENS DILUTIONS	ANIMALS
No reaction in 1:10	♂376A.2, ♂398A.1, ♀418A.1
1:10	♂376A.1, ♂392A.1
1:100	♂393A.1, ♀422A.1*
1:15,000	♂387A.1*

* Received ten calf lens injections altogether, the last one April 3, 1931.

DESCENDANTS OF TREATED ANIMALS

Altogether 177 normal-eyed and no defective-eyed offspring were born to the treated rabbits of the fourth experiment. For various reasons a number of these animals were kept for breeding purposes. The prime object, however, was not to determine whether or not defective-eyed offspring would appear in later generations. Nevertheless it may be of interest to give some of the details.

In order to keep the relationship between the different generations clear it may be helpful to use the terms P_1, F_1, F_2, etc., applying P_1 to the gen-

eration that received the injections. The 177 animals mentioned above would then be the F_1 generation. To produce the F_2 generation two different types of mating were used. In the first, the F_1 animals mated were full brothers and sisters, the total offspring being ninety-three, all normal-eyed. In the second, the F_1 parents were unrelated. The total from this mating was fifty-five, also normal-eyed. The F_2 generation thus consisted of 148 normal-eyed animals.

The number of F_3 animals produced up to the present is small, being only nineteen, all normal-eyed. Fourteen other animals were produced from parents, one of which was an F_2 animal and the other an F_3. These also were normal-eyed.

Twenty-six additional animals were obtained from matings between F_1 and F_2 individuals. Here there were three offspring with defective eyes, the remaining twenty-three being normal. Since the above three are the only defective-eyed descendants of the treated stock, the circumstances surrounding their production should be given more in detail.

They all belonged to the same litter, 536A, and they comprised the entire litter. The date of birth was March 19, 1932. When comparatively young they left the nest box and before long were emaciated. To all appearances they had a cold which was manifested by the presence of dried pus on the eyelids. We occasionally have had animals showing these symptoms, and with this one exception the recovery has been complete.

Upon examination at less than one month of age, it was found that at least one eye in each animal was abnormal. One member of the litter, ♀536A.3, died April 24, 1932 of coccidiosis. Her entire left eye, including the lens, was opaque. She was not photographed.

The other two animals, ♀536A.1 and ♂536A.2, have been photographed (May 14, 1932), and are shown in figure 1. The right eye of ♂536A.2 is normal, while the left is entirely missing. What appears to be a defective eye in the photograph is the inner side of the two eyelids. The condition of the eyes of ♀536A.1 is apparent from the photographs. She was entirely blind in both eyes, and early in November, 1932, died of coccidiosis, after having shown symptoms of disease throughout life. The male, 536A.2, on the other hand, at the end of a year was still apparently in good health.

The eye defects all appeared to be due to infection, and, since the mother (♀513A.4) later was shown to have coccidia in her feces, it was thought that coccidial infection of the eyes might have been the causative agent. On April 29, 1932, the pus and other material in the eye socket of ♂536A.2 was carefully examined by the junior author, but no coccidia were found. The type of infection therefore is not definitely known.

Female 513A.4, the mother of litter 536A, was afterwards bred to the

same male, 454A.1, and produced a litter of three, all normal-eyed. Two of these died young, one of them apparently of coccidiosis. The remaining animal, ♀544A.1, was bred to ♂536A.2, and gave birth to a litter of seven, all of which died within a week after birth because of neglect by the mother. She was for this reason discarded.

Although there does not seem to be much question but that the eye defects of litter 536A were due to infection, it has been deemed advisable to give the pedigree of the litter. The mother's (♀531A.4) parents (♂474A.7 and ♀474A.8) were full brother and sister and their parents were ♂398A.1 and ♀421A.1, both of which had received calf lens injec-

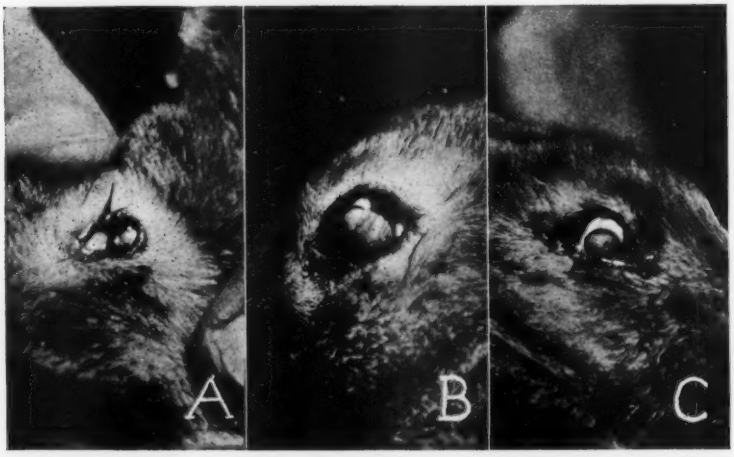

FIGURE 1.—Eye defects believed to be produced as a result of infection by disease-producing organisms. *A*, empty eye-socket of ♂536A.2. *B*, left eye of ♀536A.1. *C*, right eye of ♀536A.1. The two animals are litter-mates. Their paternal grandparents and maternal great-grandparents received calf lens injections.

tions. Male 454A.1, the father, was an F_1 and his parents were ♂392A.1 and ♀424A.1. There is nothing unusual about the pedigree except the fact that one of the P_1 animals, ♀421A.1, had received ten lens injections and that her offspring, ♂474A.7 and ♀474A.8, came from a litter that was born nine days after the tenth injection.

DISCUSSION

It is evident that although we have used all of the three methods employed by GUYER, and have obtained at least as many offspring from treated parents as he, his and our results differ materially in regard to the percentage of defective-eyed offspring produced.

It may be of value to speculate on the possible causes for this difference. We are satisfied from our own experiences that there are at least two other causes for eye defects. One of these is heredity, which is responsible for many of the apparently spontaneous cases. Evidence on this point was given in our 1931 paper. The other is direct infection by disease-producing organisms. Evidence in regard to the latter, unsatisfactory in some respects, is given in the present paper. These two causes combined, however, have not produced a single eye defect in our F_1 animals, and only a very small percentage in the later generations, but they have been responsible for all the defects that were produced. GUYER considers that heredity plays a relatively unimportant part, as applied to his material, and, so far as can be ascertained, does not take infection into consideration at all.

It seems reasonable to us to assume that if the blood of a pregnant animal contains an appreciable amount of lens antibody (as measured by its precipitin reactions) throughout pregnancy, and that if the placenta does not obstruct to any extent the passage of the antibody from the mother to the fetus, there should always be at least some offspring born with precipitated, or milky-looking, lenses. Since it seems impossible to account for all the eye defects produced by GUYER by ascribing them to either heredity or infection, and since we are unwilling to ascribe the eye defects of any of our animals to effects produced originally by lens antibodies, the most satisfactory manner of reconciling the two results is to make the assumption that some of the placentas of GUYER's animals were permeable to the antibody while none of ours were.

The difference in placental permeability could conceivably be due either to heredity or to environment. It does not seem probable, however, that GUYER should have a fairly plentiful supply of rabbits with hereditary placental permeability while we and other workers, such as HUXLEY and CARR-SAUNDERS (1924), should not have a single animal of this description. Hereditary permeability therefore is evidently ruled out, and the only alternative is the assumption that environment is the cause. The environment of an animal consists not only in the feed, but also in the method of handling (in this case particularly the manner in which it is carried while pregnant) and the temperature, humidity, et cetera. Nothing is known concerning the relation of these environmental factors to placental permeability, and for that reason it is perfectly safe for the present at least to assume that a causal relation of some sort does exist. The conclusion to be drawn from the above statement is that GUYER's animals must have been kept under environmental conditions differing from those under which the animals of other workers were kept.

SUMMARY

1. Sixty-three out of a total of sixty-eight rabbits received five weekly injections (either subcutaneous or intraperitoneal) of calf lens material weighing slightly more than 1 gram per injection.

2. Ten days after the fifth injection, titrations were made for antibody content. The undiluted serum of twenty-eight of these animals caused precipitation in calf lens dilutions of less than 1:5,000; twenty-one in dilutions ranging from 1:5,000 to 1:10,000; and the remaining fourteen in dilutions of over 1:10,000 and up to 1:25,000. The antibody content of seventeen of the above animals was also determined by titration against rabbit lens. No appreciable difference in titer was obtained.

3. Additional titers were taken of six animals on the sixty-sixth day after the last (fifth) injection, and of twenty-six after forty-five days had intervened. Out of this total of thirty-two, twenty-one had already lost all their antibody, while the remaining eleven caused precipitation only in low dilutions, the highest being 1:100. No correlation was found to exist between the amount of antibody (as measured by the precipitin titer) and the length of time the antibody remains in the animal.

4. The results reported above were obtained in four separate experiments. In the first three experiments nearly all the rabbit females died before reproducing. In the fourth experiment there were twenty-four females each of which produced one or more litters. The individuals in these litters totaled 177, and they were all normal-eyed.

5. Details of fourth calf lens experiment. All of the rabbits, including six males, received the weekly injections on the same days, but the breeding of the females was spread out in such a manner that three produced seventeen offspring after having received the first of the five injections less than a week before parturition. Three females produced fourteen young after having received the first two injections previous to parturition. Five females produced twenty-eight young after having received the first three injections, and four produced sixteen after having received the first four injections. Twenty-three offspring were born to three females which had received their fifth injection within two weeks of parturition. Twelve females were bred from seven to thirty-two days after having received the fifth injection to males which had received five injections, and gave birth to fifty-six offspring.

Three female rabbits of the fourth calf lens experiment received ten weekly injections instead of five. They were mated within a day of each other to males which had received five injections. The three litters, containing sixteen individuals, were born about a week after the mothers had been given the tenth injection. One of the above mothers was bred again

to a male which had received ten injections. Copulation took place 131 days after the tenth injection had been made. The purpose of this last mating was to determine whether the lens antibody had had any direct effect on the germ cells of the parents. The seven offspring were normal-eyed, however, thus bringing the total number, as previously mentioned, to 177, all normal-eyed.

For clearness, the treated animals of the fourth experiment have been called the P_1 generation and their 177 offspring the F_1 generation. The F_2 animals consisted of 148 individuals, all normal-eyed. As yet there are only nineteen F_3 animals; these also were normal-eyed.

The only defective-eyed animals of the fourth experiment were found in a litter of three produced by an F_2 female mated to an F_1 male. All three had marked defects, but there is good reason for believing that infection of the eyes by disease-producing organisms was the cause.

6. Since our results differ materially from those of GUYER, it is proposed that this may be due to a difference in placental permeability to lens antibody. The possibility of this difference being due to heredity is ruled out because it seems improbable that GUYER, who used animals from various sources, should consistently have obtained those with hereditary permeability, while other workers did not procure a single animal of this type. The only alternative is to assume that, due to as yet unknown environmental influences, the lens antibody passed through the placenta more easily, or oftener, in GUYER's animals.

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ALLELES[1] OF THE MALLARD PLUMAGE PATTERN IN DUCKS[2]

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Very little information has been published on the genetic composition of the various breeds and varieties of ducks. The present investigation was started to study the inheritance of these varietal differences. The Standard (American Poultry Association 1930) varieties, Rouen and Gray Call, have a plumage pattern similar to that commonly found in their wild ancestor, the Mallard. The most outstanding difference between these two Standard varieties is in size rather than color, the Rouen being selected for large, and the Gray Call, small size. The term "mallard" is used to designate this pattern type wherever it occurs; "Mallard" (capitalized) refers to the wild duck or stock reared from it in captivity. This mallard plumage pattern was used as a starting point since it represents the wild-type. It was early noted that variations occur in this pattern which are rather constant in expression. A series of three patterns produced by three allelic genes, all of which give an adult color pattern approaching the generally accepted wild-type, are herein discussed.

Early in the work the stock consisted of partially domesticated Mallards. Some of these were obtained through the courtesy of the State Conservation Commission, others consisted of stock bred by the Poultry and Genetics Departments of the UNIVERSITY OF WISCONSIN, and a few were secured from local breeders. Later White Pekins were used, which proved to be genetically mallard in plumage pattern; that is, having this pattern as a cryptomere under the recessive white. For sake of clearness, as in a previous report (JAAP 1933) the pure white segregates have not been included in the material presented.

DESCRIPTION OF TYPES

The three down color types and their corresponding adult patterns have been designated as mallard, dusky mallard, and restricted mallard. The following is a description of these types.

The "mallard" pattern

The dorsal surface of mallard ducklings (figure 2A) is of a dark color closely approaching olivaceous black. Four dull yellow spots (see figure 1),

[1] In this and following issues of GENETICS the use of "allele" for "allelomorph" will be an editorial policy.

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irregular in outline, are invariably found on the back. These may vary somewhat in brightness but their location is constant. The sides of the head are dull yellow, with one or two dark ocular stripes extending from the base of the bill to the dorsal dark area. The variability in these dark ocular stripes is considerable. Mallards always have at least one of these stripes and this is the main character used in classification. When there is only one stripe it extends across the eye and may or may not unite with the dorsal dark area. The second of these stripes may appear as a mere spot near the back of the head and another at the base of the bill where the two mandibles meet. All gradations are found between this latter type and those having two complete ocular stripes. On some ducklings the two stripes may fuse below and just posterior to the eye. On others,

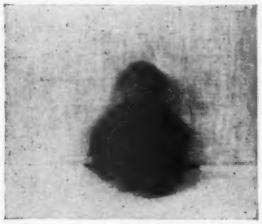


FIGURE 1.—Dorsal view of "mallard" duckling showing the location of the four light colored spots on the back.

neither of the oculars may unite with the dark area of the dorsal surface. The ventral surface, from the base of the bill to a point where the white neck ring appears on the adult male, is yellow in color. The down here is very short, which makes it impossible to distinguish any difference between the surface and undercolor. The same is true for the ventral surface of the wings. The remainder of the ventral surface is also dull yellow but the down here is characterized by a dull black to slate undercolor. On some ducklings the dark undercolor may extend out to the surface, giving the region a mottled appearance. The upper surface of the bill, the legs and the feet, are dark horn-color, while the under surface of the bill shows only the yellow carotinoid pigment.

In adults (see figure 4) "mallard" conforms very closely to the description as outlined by the American Standard of Perfection (American Poultry Association 1930). Both males and females of the stock used, however, are usually of a somewhat lighter shade owing to a reduced size of the dark brown markings as compared with the ideal feather. The precise regularity of feather pattern described in the Standard seldom, if ever, occurs. The color of the upper mandible of the drake, and of that part of

the duck's bill not covered with dark pigment, varies considerably. It ranges from greenish yellow through dark green to leaden. Narrow white tipping on the claret breasts of the males is common and, in extreme cases, may make the median area appear white. The sides of the head on females and the first feathering of both sexes in the head region tend to vary with the down color. The dark portion of the feather pattern is usually lacking

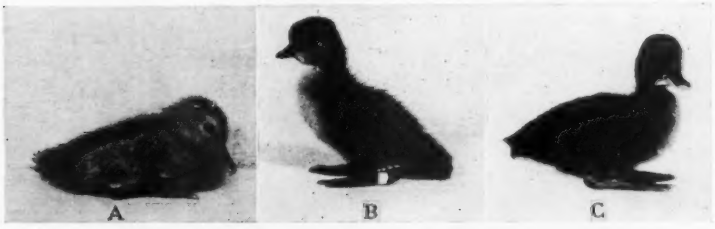


FIGURE 2.—(A) "Restricted," (B) "mallard," and (C) "dusky" ducklings.

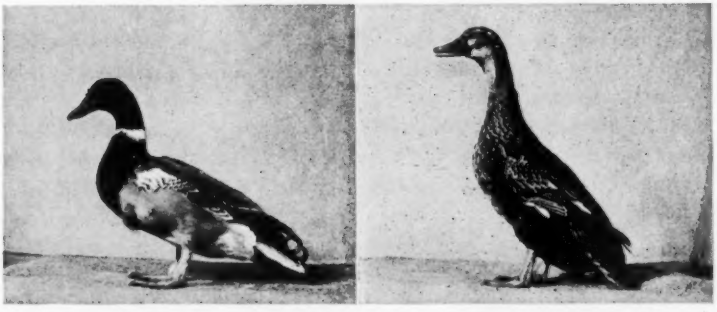


FIGURE 3.—Adult "restricted" male and female. Note the white on the dorsal wing surface of the male. The white on the female's wing is limited to the wing front, which is nearly covered by body feathers. The feathers on the remainder of the dorsal wing surface have a wide lacing of buff.

for some distance from the base of the bill on the ventral side of the neck of females. Several "mallard" females, however, have been recorded in which this area has the dark feather pattern.

Dusky "mallard"

The "dusky mallard" or "dusky" pattern has been obtained from the Mallard, White Pekin, Buff, and black breeds. Dusky ducklings (figure 2C) exhibit a uniform olivaceous black over the entire dorsal surface, gradually becoming lighter to a dark-olive-gray on the ventral side. There

are no dull yellow spots on the back of dusky, this being a constant difference from mallard. The ventral surface of the wing is deeply pigmented. The bill, legs and feet have a dark color which always shows a distinct brownish cast. As in mallard the under surface of the bill usually lacks the dark color, but a small spot may appear at the tip. Dusky ducklings, as evidenced by their plumage pattern when adult, have been described

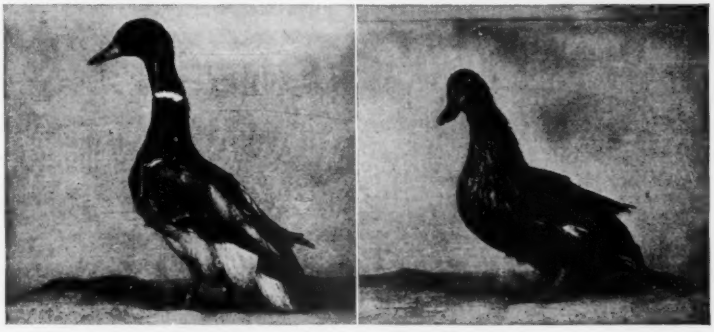


FIGURE 4.—Adult "mallard" male and female.

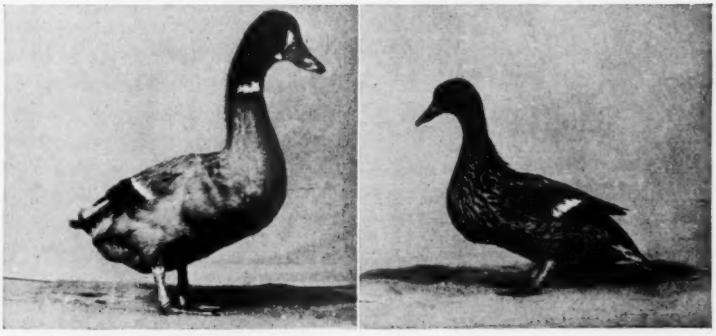


FIGURE 5.—Adult "dusky" male and female. Note the absence of claret-brown on the breast of the male and the irregular white spot on the throat. This white spot occurs equally often on females as males. The uniform coloration of the head is characteristic of the female.

in the literature as "*vrais petit negrillons*" by ROGERON (1903), "sooty black" by FINN (1913), and "coal black" or "freak" by PHILLIPS (1915, 1921). A "sooty aberrant" type described by PUNNETT (1932) was probably dusky.

Adult dusky drakes usually deviate from mallard by a complete ab-

sence of the white neck ring. If white is present in the neck (figure 5) it is not a secondary sexual character and always appears in the first feathering of both sexes. The claret breast of most dusky males is reduced to a very small amount in a region close to where the white neck ring is located on mallard. Quite a few individuals, however, show even more claret than is minimum for mallard. The iridescent bar on the secondary wing feathers is invariably obscured and the secondary coverts have more white (figure 5). Dusky females show no sign of the light ocular stripes usually present on mallard ducks. Though the females are usually somewhat darker in shade, some duskies have been raised which are even lighter in color tone than the darkest of the mallards. The only part of the plumage in which dusky invariably differs from mallard is the ventral wing surface. In dusky this region is always pigmented. Furthermore, the feather pattern is similar to that of the body, while in mallard it is white.

GOODALE (1911) is the only author who has noted the pigmented ventral wing surface of dusky, but he failed to associate it with the dusky down pattern. The only uniformly colored ducklings raised by GOODALE were reported to have been black when adult. The association of the uniform head color of the females, and the obscured wing speculum of both sexes, with a pigmented ventral wing surface in the adult is shown by him in tabular form along with some other variations. The males described by PHILLIPS (1915, 1921) had very little, if any, claret-brown on the breast, while FINN (1913) observed a gradation from a mere trace to an amount of claret similar to mallard. GOODALE (1911) has recorded one male without and one with claret-brown in the anterior breast region. ROGERON (1903), FINN (1913), and PHILLIPS (1915, 1921) report an absence of the white collar, or neck ring, while both the males given in the table presented by GOODALE (1911) had a white neck ring.

Restricted "mallard"

The term "restricted" has been given to the other variant since, in the down, the dark area of the dorsal surface is restricted to a patch on the head and tail (figure 2A). The remainder of the dorsal surface is dull yellow with a dark undercolor. The ventral surface, bill, legs and feet are the same as described for mallard. About the only variation occurring within this type is in the general color shade of the duckling. This is due to the extent to which the dark color at the base of the down feather extends out towards the tip. In some cases the dark tail area may extend a short way up the back, gradually blending into the dorsal surface color.

Restricted mallards as adults (figure 3) are almost indistinguishable from mallard. The only constant difference is in that area designated by the Standard (American Poultry Association 1930) as the wing front and

bow. In "restricted" this area always shows considerable white in the males, the white appearance being due to partially white rather than to completely white feathers. Restricted females have white spotted feathers on the wing front, and the wing bow is much lighter in color than mallard or dusky owing to a very wide lacing of buff. This restricted type of mallard plumage coloration has not, to the writer's knowledge, hitherto been described in the literature.

GOODALE (1911) obtained from Rouen-Pekin crosses a new type of down pattern in about one-half of the progeny, the remainder having the typical Rouen coloration. These were described as "dull yellow, very different from the Pekin color, and with an under color of dull black which usually came to the surface on the wings and tail." The adult males had much claret on the dorsal side of the lower neck and the more anterior parts of the dorsal wing surface had an admixture of white and rufous. One F_1 male with color pattern similar to that of the Rouen was raised. Since the down colors of each individual were not reported it is not known whether the above mentioned types of down and adult colors correspond. FINN (1913) records that three yellow ducklings with a dash of black on the crown were observed in the progeny of a Mallard. These ducklings as adults were described as being pale brown with dark markings. No inheritance studies were undertaken on either of these two types.

EXPERIMENTAL

The following material, except where specified, is gathered from matings in which both parents are known. The exceptions consist of pen matings of one male with two or three females. Since the adult and duckling classifications correspond, the records presented in the following tables are the down color descriptions. By this procedure many of the ducklings could be disposed of at hatching and thus make it possible to increase the number classified. The letter M designates the wild-type (mallard) and m^d the recessive, dusky. Since restricted is dominant and allelic to the others it is designated by the symbol M^R.

The relation of dusky to mallard

The results of the matings involving only mallard and dusky are given in table 1. In mating 2 a dusky female mated to an unrelated mallard male produced 34 mallard ducklings. Fifteen ducklings were described from the reciprocal cross (mating 6), all of which were mallard. In the F_2 generation (Y18-1, U354, 29, 36, and 59) there were 97 mallard to 43 dusky, a deviation of 8 from the expected 3:1 ratio, with a probable error of 3.45. Matings 9, 41, and P41 are backcrosses of F_1 progeny to dusky. These produced 90 mallard and 104 dusky ducklings, a deviation of 7 ± 4.69 from

the expected 1:1 ratio. Dusky segregates mated together, in matings 3, 19, 21, 24, 52, 62, and 67, produced 253 ducklings, all of which were duskies. These matings demonstrate a single gene difference between mallard and dusky. PHILLIPS (1915, 1921) also reported dusky a simple recessive to mallard.

TABLE 1

	MATING NO.	TYPE OF MATING MALE × FEMALE		OFFSPRING MALLARD	OFFSPRING DUSKY
(a)	2	MM	m^dm^d	34	0
	6	m^dm^d	MM	15	0
	Total			49	0
	Expected			49	0
(b)	Y18–1	Mm^d	Mm^d	47	17
	U354	"	"	4	3
	29	"	"	16	10
	36	"	"	13	7
	59	"	"	17	6
	Total			97	43
	Expected			105	35
	Deviation	8 ± 3.45			
(c)	9	m^dm^d	Mm^d	51	41
	41	m^dm^d	Mm^d	29	47
	P41	Mm^d	m^dm^d	10	16
	Total			90	104
	Expected			97	97
	Deviation	7 ± 4.69			
(d)	3	m^dm^d	m^dm^d	0	44
	19	"	"	0	43
	21	"	"	0	40
	24	"	"	0	36
	52	"	"	0	57
	62	"	"	0	23
	67	"	"	0	10
	Total			0	253
	Expected			0	253

With the exception of matings 59 and P41, the birds used in table 1 were entirely of Mallard origin. Therefore, as ROGERON (1903), FINN (1913), and PHILLIPS (1915) have found, the dusky gene is present in some races of the wild Mallard. Mating 59 is a mating of two buff ducks obtained from a local fancier. Both mallard and dusky pattern appeared in the young. Since none of these were raised to feathering the adult patterns were not checked. Both parents in this mating must accordingly have been heterozygous for dusky. The male used in mating P41 is the same buff drake as in 59 and the female was an F_1 of a Pekin-dusky cross (mat-

ing P8, table 3). These two matings demonstrate that Buff ducks may have either the gene M or m^d, or both. Since dusky ducklings were produced in mating P8, and in other Pekin-Mallard matings, it is evident that White Pekins also may carry both mallard and dusky. Data not presented here show, in the same manner, that black ducks (a breed known as East India) may also carry both these genes.

TABLE 2

	MATING NO.	TYPE OF MATING MALE×FEMALE		OFFSPRING RESTRICTED	OFFSPRING MALLARD
	P1	M^RM^R	MM	130	0
	P5	M^RM^R	Mm^d	91	0
	P16	M^RM^R	MM	10	0
(a)	P31	Mm^d	M^RM^R	31	0
	P37	M^RM^R	MM	12	0
	Total			274	0
	Expected			274	0
	P9	M^RM	M^RM	34	15
	P11	M^RM	M^RM	15	7
	P12	M^RM	M^RM	23	8
	P13	$M^R(m^d)$	M^RM	13	4
(b)	P15	M^Rm^d	M^RM	4	1
	P17	M^Rm^d	M^RM	18	10
	P36	M^Rm^d	M^RM	22	7
	Total			129	52
	Expected			135.75	45.25
	Deviation	6.75 ± 3.93			
	40	m^dm^d	M^RM	27	28
	P6	M^Rm^d	MM	19	22
	P10	Mm^d	M^RM	16	26
	P14	M^Rm^d	MM	3	3
(c)	P30	m^dm^d	M^RM	12	12
	P32	Mm^d	MM	31	21
	P44	M^RM	MM	21	15
	Total			129	127
	Expected			128	128
	Deviation	1.5 ± 5.38			

The relation of "restricted" to mallard and dusky

The data on the heritable relation of restricted to mallard and dusky are presented in tables 2 and 3. In table 2 (a) mating P1 consisted of a White Pekin male (1.6) mated with 3 Mallard females known to be MM. This mating produced 130 ducklings, all restricted in pattern. The same male (mating P5) mated to 3 females known to be Mm^d gave 91 restricted ducklings. An F_1 mallard female from another Pekin-Mallard cross was mated to male 1.6 in each of matings P16 and P37. These produced 10

and 12 colored ducklings respectively, all restricted. Mating P31 consisted of the Buff male, previously cited in matings 59 and P41, mated to a White Pekin female. The 31 ducklings described from this cross were restricted. From these matings it is evident that restricted is completely dominant to mallard and dusky and was obtained from the Pekin.

In table 2 (b) are included matings in which a ratio of 3 restricted to 1 mallard was expected. The total of 129 restricted to 52 dusky approaches the calculated reasonably well with a deviation of 6.75 ± 3.93. The females used in all of these matings and the males of P9, P11, and P12 were progeny of mating P1, and thus a further check on their genotype was provided. The male of mating P13 is a White Pekin and has not been tested further. The males of P15, P17, and P36 have all been tested further,

TABLE 3

	MATING NO.	TYPE OF MATING MALE×FEMALE		OFFSPRING RESTRICTED	OFFSPRING MALLARD	OFFSPRING DUSKY
(a)	P23	$M^R m^d$	$m^d m^d$	7		3
	P24	$M^R m^d$	$m^d m^d$	21		22
	Total			28		25
	Expected			26.5		26.5
	Deviation	1.5 ± 2.45				
(b)	44	$M^R m^d$	$M m^d$	17	14	8
	P8	$M^R m^d$	$M m^d$	19	7	7
	Total			36	21	15
	Expected			36	18	18
	Probability = .5 to .7					

proving that they were genetically $M^R m^d$. In the matings included in part (c) of this table a fair agreement with the expected equality of restricted and mallard is noted, 129 being restricted and 126 mallard. Each of the parents has been tested further in other matings which confirmed their genotype.

The relation of restricted to dusky is further analyzed in the matings given in table 3. In part (a) of this table an equality of the two types is expected. The actual numbers described were 28 restricted and 25 dusky, a deviation of 1.5 ± 2.45 from the calculated. The male of P23 was also mated in P17, table 2. Both matings demonstrated that he was heterozygous for dusky. Similar confirmation is made of the genotype of the male in mating P24, since he was used the previous season in mating P8. A ratio of 2 restricted, 1 mallard and 1 dusky is expected in both matings 44 and P8. The females of these matings were mallards known to be $M m^d$ and the male of mating 44 was from a cross of restricted by dusky and

should accordingly be $M^R m^d$. The total results of 36 restricted, 21 mallard and 15 dusky are a reasonably close approximation to the calculated 2:1:1. The probability of these deviations being due to chance alone, as measured by the X^2 method of goodness of fit, is between 0.5 and 0.7.

EVIDENCE FOR ALLELISM OF M^R, M AND m^d

The data presented in tables 1, 2, and 3 show: (1) that mallard is a simple dominant to dusky; and (2) that restricted is due either to a dominant allele or to an independent factor pair, the dominant member of which would be epistatic to mallard and dusky. A crucial mating was planned to test these two possibilities. The results are presented in the two charts on the following page.

These charts represent the expected and observed results on the basis of the two alternative postulates. On each hypothesis the original male is assigned a genotype which fits the results obtained by mating him to three known types of females. In chart 1 the female selected for the final mating received the restricted gene from her sire and mallard from her dam whereas in chart 2 the sire contributes both restricted and dusky. By mating this female to a dusky male the progeny would be half restricted and half mallard in the first case while on the second hypothesis a ratio of 2 restricted: 1 mallard: 1 dusky would be expected. Of the 55 ducklings classified from this mating, 27 were restricted and 28 mallard. Since no dusky ducklings were obtained it is apparent that the allelic and not the two factor hypothesis is consistent with the actual results.

RELATION OF M^R, M AND m^d TO WHITE SPOTTING

In a previous article (JAAP 1933) the inheritance of three types of white spotting was analyzed. Two of these types, bib (*b*) and recessive white primaries (*w*), were found to be simple recessive genes. The other type (*R*) exhibited incomplete dominance. In the homozygous condition, *RR*, white markings similar to those of the Fawn and White Indian Runner variety result. The heterozygous condition, *Rr*, is detected by the presence of white primary wing feathers when the bird is not *ww*. The alleles of the mallard pattern are distinguished in adult ducks by the presence or absence of white in certain regions of the plumage. Mallard has a secondary sexual white neck ring occurring only in the male. The ventral wing surface in both sexes is white. Dusky mallards are always colored underneath the wing and the male dusky never has a secondary sexual white neck ring. White in the neck when it does occur is present on approximately the same number of females as males. Again the only constant difference between restricted and mallard is the white spotting on the dorsal wing surface. White never occurs in this region on either mallard or dusky. In the

CHART 1

Observed and expected results of certain matings tested on the hypothesis of three allelic genes; M^R (restricted), M (mallard), and m^d (dusky). Restricted is dominant to mallard and dusky.

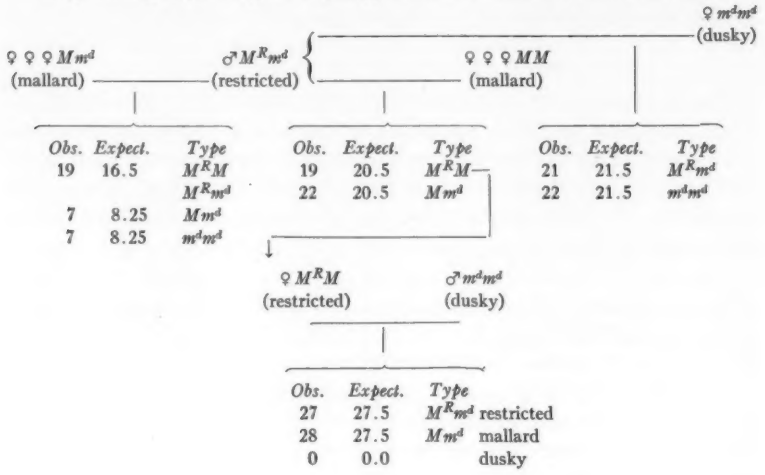
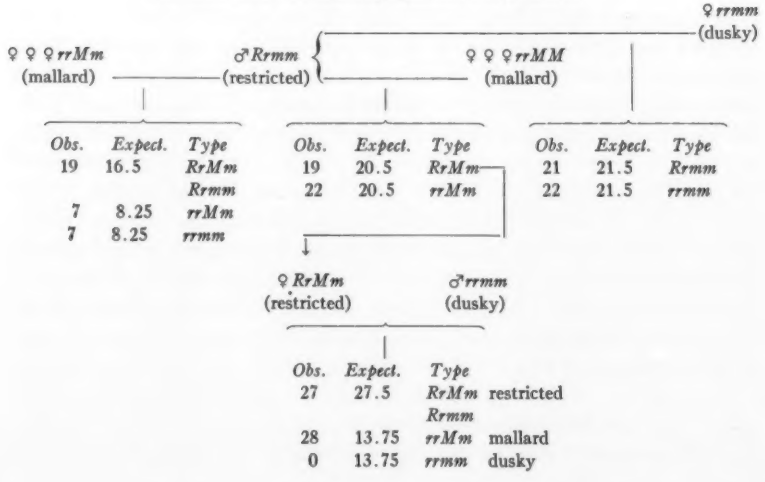


CHART 2

The same matings as in chart 1 showing that the hypothesis of two factor pairs will not fit the results obtained.

R = restricted r = not restricted M = mallard m = dusky

R is assumed to be completely epistatic over M and m.



adult restricted male there is considerable white on the wing front and wing bow. In the female also the wing front has much white, but the remainder of the wing differs from that of mallard and dusky by a restricted dark brown area and an extremely wide pencilling of light brown or buff on each feather. It is, therefore, evident that these alleles form three additional genes influencing white spotting.

A summary of the genes at present known in ducks which affect white spotting in the various parts of the body is as follows:

Section of the plumage	Genes producing White	Color
anterior breast	*b*	*B*
posterior breast	*RR*	*Rr*, *rr*
ventral wing surface	M^R, *M*	m^d
dorsal wing surface	M^R	*M*, m^d
neck ring of male	M^R, *M*	m^d
neck[4]	*RR*	*Rr*, *rr*
primaries	*RR*, *Rr*, *w*	*rr*, *W*
secondaries	*RR*	*Rr*, *rr*

SUMMARY

1. Three distinct variations of the mallard plumage pattern, namely, restricted mallard, mallard, and dusky mallard are described.

2. The down pattern of each type corresponds to a definite type of adult plumage color.

3. The inheritance of these patterns is explained on the hypothesis of three allelic genes. Restricted (M^R) is completely dominant to mallard (M) and dusky (m^d). Mallard is dominant to dusky. All the possible homozygous and heterozygous combinations have been obtained.

4. The gene m^d has been obtained from the Mallard, White Pekin, Buff, and East India Ducks.

5. Restricted was obtained from the White Pekin breed, where it was not expressed owing to the absence of the gene for the production of color in the plumage.

6. All the known genes affecting white spotting are discussed in relation to the different parts of the plumage. Restricted and mallard produce a white ventral wing surface while the dusky gene causes this region to be colored. The dorsal wing surface of dusky and mallard is colored, whereas restricted produces white spotting on the feathers. Dusky eliminates the secondary sexual white neck ring of the male.

[4] The term "neck" refers to the entire neck of the bird from the head to the body, as contrasted with the narrow white collar present on mallard and restricted males, which is designated by the term "neck ring."

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THE OPERATIONS OF A LETHAL FACTOR IN APOTETTIX EURYCEPHALUS (GROUSE LOCUSTS)[1]

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Received July 5, 1933

This paper deals with (1) the discovery in *Apotettix eurycephalus* Hancock of an autosomal lethal factor linked with those for the dominant, elementary color patterns, (2) the approximate, uniform stage of embryonic development attainable by the homozygous lethals, and (3) the apparently increased viability and probably vigor of individuals heterozygous for the lethal over sibs homozygous for its normal allele.

Specimens of *A. eurycephalus* have been brought in from the regions of Tampico, Mexico, Houston and San Antonio, Texas, annually, or biennially, over a period of twenty-two years, and bred in the greenhouse laboratory. The accumulated genetic and cytological evidence seems to justify the assumption that the closely linked factors for the thirteen dominant, elementary color patterns are all located along one, the smallest of the six pairs of autosomes (NABOURS 1925, 1929; NABOURS and ROBERTSON 1933). It is to be shown in this paper that the factor for lethal has a definite place also on the first pair of chromosomes (see chart 1).

Special obligations are gladly acknowledged to IVA LARSON, FLORENCE STEBBINS and L. MARJORIE DEAN for valuable technical aid, suggestions and criticisms.

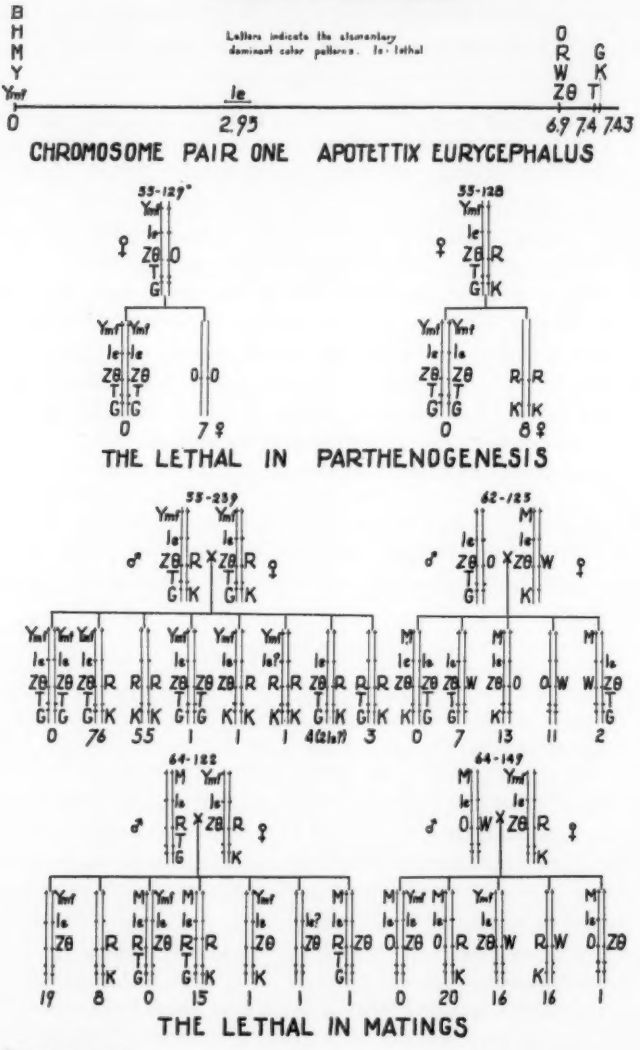
THE DISCOVERY OF THE LETHAL

In the summer of 1929, two sisters, one with the dominant color pattern combination *YmfZΘTG/O* and the other *YmfZΘTG/RK*, reproducing parthenogenetically in separate cages, gave 7 *O/O* females and 8 *RK/RK* females, respectively, and none of the ordinarily expected combinations *YmfZΘTG/YmfZΘTG*. Following these results, the other specimens of this stock were conserved, so that, in 1930 and subsequently, many matings were made which further definitely indicated the presence of a factor for lethal in a particular position on the chromosome bearing the dominant color pattern combination *YmfZΘTG*.

The chromosome bearing the lethal, since it was marked by the genes for the color patterns, could be traced back through the pedigrees to a female carrying the factors *Ymf(le?)/(le?)ZΘ* in which there was a crossing over to produce the *YmfleZΘ* (*T* and *G*, to complete *YmfleZΘTG*, were added subsequently by crossing over). Each of these homologs, *Ymf(le?)*

[1] Contribution 152 from the Department of Zoology.

CHART I THE LETHAL IN PARTHENOGENESIS AND IN MATINGS



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and (*le*?)$Z\Theta$, was further traced back to specimens collected at West End Lake, San Antonio, Texas, in October, 1926. The $Z\Theta$ had been paired homozygously several times in the laboratory without disclosing a lethal, but the *Ymf* had not been so tested. Therefore the lethal factor was probably brought in from nature linked with that for the *Ymf* color pattern. However, there had been remote opportunity for linking the lethal from other sources with *Ymf* by crossing over; or it might have originated by mutation at some time in the laboratory.

In the females carrying the lethal, crossovers have occurred between the locus, or possibly the extremely closely linked loci, of the color pattern genes *B*, *H*, *M*, *Y* and *Ymf*, on the left, and the locus, or closely linked loci, of *O*, *R*, *Z*, Θ and *W*, approximately 6.9 points to the right. In the 119 of these crossovers that were tested 70, or 59 percent occurred between the lethal and the locus, or loci of *O*, *R*, *Z*, Θ, *W*; and 49, or 41 percent, between the lethal and the *B*, *H*, *M*, *Y*, *Ymf* locus, or loci. These crossings over therefore show not only that the lethal is on the same chromosome pair with the genes for the elementary, dominant color pattern, but that it is markedly nearer the latter group (see sample matings and chromosome map, chart 1).

THE EMBRYONIC STAGE ATTAINABLE BY THE HOMOZYGOUS LETHALS

Sufficient numbers of heterozygous lethals were bred to ascertain that approximately one-fourth of the offspring, as determined by the color pattern combination expectations, never appeared in the records which were made in the third to fifth, or even earlier, instars. However, it was not known how far the double lethal embryos developed, if development was ever initiated. Accordingly the batches of eggs were removed from the ground soon after the newly hatched offspring were observed to have emerged. It was quite easy to distinguish the shells of the hatched, from the unhatched eggs, and the former closely approximated the numbers of the newly hatched offspring in each case. From 3766 such eggs, 2652 offspring, and nearly the same number of shells, were counted, and there were 1090, or 28.95 percent, unhatched. The small discrepancy of 24 is attributed to errors in counting egg shells and offspring. From the group of non-lethal bearing, control matings, under similar breeding conditions, there were 1899 offspring, and approximately the same number of shells, and 102 or 5.07 percent, of unhatched eggs among 2012 eggs counted. Such unhatched eggs did not usually contain embryos, development probably never having begun. These data show then that the homozygous lethal is responsible for the failure to hatch of at least 23.88 percent of the eggs from the heterozygous lethal bearing parents.

Nearly all of the unhatched eggs of the heterozygous lethal parents

removed from the ground, after the viable sibs had emerged, showed advanced embryonic development. The pigmented eyes could be noted through the shells. After preparation, many of the embryos were removed from the shells, some of them preserved whole, and others sectioned. No irregularities in development, nor deformities, could be detected from a careful study of the whole embryos, and the stained sections of these homozygous lethal embryos, as compared with controls killed four or five days before time for hatching. In both controls and double lethals at this stage blastokinesis was complete, but the appendages were immature. The mesenteron contained a considerable mass of yolk and was thin-walled and the mesoderm around it was loosely arranged. The dorsal organ or "heart," the Malpighian tubules and the trachea were plainly visible. Very little muscular development was evident. The caeca of the anterior end of the mesenteron were still solid and only slightly differentiated.

It remains to be ascertained whether the embryos which never hatch develop normally until four or five days before time for emerging and then die suddenly, or the whole or part of the embryonic progress is slower so that they are incompletely developed and die at about the regular time for hatching. There is then the alternative that the double lethal embryos may develop more rapidly at certain stages and then slow down till they are found to be actually behind the normals at the time for hatching. It is not thought that the death of the double lethals occurs long before the time for emergence of the viable sibs, because the tissues are still in good condition, though they remain so for only a few hours thereafter. These questions are reasonably within the sphere of experimentation, and it is hoped that answers will be forthcoming.

THE STIMULATING EFFECT OF THE HETEROZYGOUS LETHAL

The presence of the lethal on only the one chromosome of the pair does not make any, at least striking, difference in the appearance of the individual. There is no sex-limited effect, as the sex ratio is normal. However, the tabulation of 9657 offspring of lethal-bearing parents, marked by the genes for the dominant color patterns, recorded in the third to fifth instars, revealed 6560 heterozygous lethals, and 3097 homozygous nonlethals. The former were therefore 122 individuals, or 3.91 probable errors, more viable, or above the expected ratio of 2:1. In 7607 offspring from parents one only of which carried the lethal, 3969 contained it, while 3638 did not. This comprised a difference in favor of the viability of the heterozygous lethals of 165.5 individuals, or 5.63 probable errors.

The question of control now arises, and we have concluded that there is possibly none available further than to check the heterozygous lethal with its homozygous normal allele, which is probably sufficient. However,

the following appended table indicates a similar comparison of the factors for the heterozygous *T*, *G*, and *K* color patterns with their respective homozygous alleles.

TABLE 1

The comparative viability of heterozygous lethals, homozygous non-lethal sibs, and controls.

TYPE OF MATING	NUMBER OF HETEROZYGOTES	NUMBER OF HOMOZYGOTES (+/+)	DEVIATION FROM EXPECTED RATIO	$\frac{D}{PE}$
$\frac{le}{+} \times \frac{le}{+}$	6560	3097	122.0	3.91
$\frac{le}{+} \times \frac{+}{+}$	3969	3638	165.5	5.63
$\frac{T}{+} \times \frac{+}{+}$	6001	5985	8.0	.22
$\frac{K}{+} \times \frac{+}{+}$	4914	4781	66.5	2.003
$\frac{G}{+} \times \frac{+}{+}$	4382	4329	26.5	.84

Another set of data, including those of *T*, *G* and *K*, of table 1, was compiled at random from the record books. From 58,475 offspring of heterozygotes with dominant color patterns, or closely linked groups of dominants, mated with the normal recessives, or alleles, there were recorded 29,345 heterozygotes to 29,130 homozygotes, a difference of only 107.5 or 1.32 probable errors.

If these data are available as a control, of which we are in some doubt, then the increased viability of the heterozygous lethals over the sibs homozygous for the allele, is even more outstanding. Regardless of the controls in question, it seems that the lethal in the single dose significantly improves the viability, and that in the double dose it causes death at the stage 4–5 days before time for hatching.

A principle of rather wide implication and importance may have come to light through this discovery which may not be new, although we have been unable to find an account of a previous observation of exactly the same kind. It is conceivable that the effect of the lethal, however it operates, is to aid viability and vigor when there is not so much (only half) of it, and discretely to limit life to the stage 4–5 days before hatching when there is too much (a homozygous dose) of it. It may not be unreasonable to suggest that factors of this kind are sometimes responsible for hitherto unexplained variations in viability, vigor, and longevity in foetal as well as postnatal stages of domestic animals and man. Certain races noted for their marked vigor and viability, yet low in prolificity, may be thus bionomically influenced.

SUMMARY

1. An autosomal lethal factor in *A. eurycephalus* has been found to be located between the locus, or loci of those for the dominant elementary color patterns, *B*, *H*, *M*, *Y*, and *Ymf* and the locus, or loci, for those of *O*, *R*, *Z*, Θ and *W*, and somewhat nearer the former.

2. The embryos carrying the homozygous lethal develop apparently normally till within 4–5 days of hatching, and then invariably die.

3. The individuals heterozygous for the lethal survive better than their sibs homozygous for its normal allele. The effect of a double dose of the lethal is to cause death at a uniform embryonic stage, and the single dose stimulates in some way to greater viability and probably vigor.

4. It is suggested that such lethal factors may be quite numerous, some of them causing death, either pre- or postnatal, when they are in homozygous quantity, but giving increased size, vigor, and general viability when they are in heterozygous amounts, and that they are probably also present and thus operating in domestic animals and man.

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FACTORS AFFECTING BIRTH WEIGHTS OF SWINE[1]

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Received July 6, 1933

INTRODUCTION

The large variability in birth weights of mammals, particularly of multiparous mammals, suggests that many factors or conditions may be responsible for these differences. The present paper presents estimates of the relative importance of some of those things which cause the birth weights of swine to vary.

MATERIAL AND METHODS

The data were taken from the breeding and farrowing records of the Animal Husbandry Section of the IOWA AGRICULTURAL EXPERIMENT STATION during the years 1915 and 1917 to 1930 inclusive. The data were collected in connection with other experiments[2] not planned expressly to make this study possible. Yet no practice was followed which seems likely to have affected the birth weights in a way which would have invalidated this analysis.

The data analyzed are the birth weights of 506 litters containing 3639 pigs, all of which were produced by gilts about one year old at the time of farrowing. The time of weighing ranged from immediately after birth in some cases to a little more than twelve hours after birth in a few cases. Some pigs had suckled before being weighed but others had not. All litters were farrowed in the months of April and May, except nine in June, one in July and three in August. The breeds included purebred or high grade Poland Chinas, Tamworths, Hampshires and six different crosses. Over 71 percent of all these litters were purebred or high grade Poland Chinas.

To determine the importance of each factor affecting birth weight, FISHER'S (1930) method of analyzing variance was used. The percentage of the variance which disappears when a certain variable is held constant may be used to express the closeness of the relation between that variable and birth weight (table 3). For those accustomed to think in terms of correlation coefficients, it is easy to understand such a proportion as equivalent to a squared primary correlation coefficient between birth weight

[1] Journal Paper No. J 116 of the IOWA AGRICULTURAL EXPERIMENT STATION, Ames, Iowa.

[2] Dr. JOHN M. EVVARD and Professor C. C. CULBERTSON were in charge of this work and at different times the immediate responsibility for collecting these data rested on C. C. CULBERTSON, RUSSELL DUNN, C. F. BASSETT and W. E. HAMMOND.

and the variable which is held constant, provided the relations are rectilinear. In this study, the uneven distribution of the variables from year to year makes it impossible to separate the entire variance in birth weight completely into independent components ascribable to each of the variables or causes. When each of the variables in turn is studied, taking out the effects of only one or two at a time, approximate estimates are obtained of the importance of each variable as a cause or indicator of variance in birth weight, but each such estimate includes some of the variance which is jointly caused by the variable being considered and by other variables correlated with that one (table 4).

Because pigs born dead may have died an uncertain number of days before birth, it seemed best to analyze separately the data for all pigs farrowed and the data for only those pigs born alive.

FINDINGS

Sex ratio

Although not essentially related to the scope of this report, a discussion of the frequency of the different sex combinations seems of sufficient interest to be included here. McPHEE (1927) reported for 17290 pigs from several experimental herds, a sex ratio of 52.0 percent males. He found also that herdbook data from the Chester White Record and from the Duroc Jersey Record showed significant departures from the expected frequencies of the different sex combinations. Data collected from the experimental herds of the UNITED STATES DEPARTMENT OF AGRICULTURE however were in agreement with the expected values obtained by expanding the binomial $(p+q)^n$.

In the population of 3639 pigs of the IOWA STATION the sex ratio is 51.1 $\pm$.6 percent males. The probabilities that the distributions of sex combinations are random in each litter size for litters of 4–10 pigs are .29, .21, .31, .09, .08, .08, and .67 respectively. These values for P are still within what are usually considered the limits of significant departures from expectation. When these seven probabilities are combined by adding the χ^2 obtained for each litter size, the resulting probability that the distribution of sex combinations through all these litter sizes is random becomes almost exactly .05. However such departures from expectation as do exist, show no consistent excess or deficiency of litters which are predominantly of one sex. For example in the 9-pig litters the principal deficit is in litters containing 3 males while the principal excess is in litters containing 4 males. Hence it is concluded that the frequency of sex combinations was probably a chance affair conforming to the binomial $(p+q)^n$ in which $p=.511$.

The χ^2 test shows a probability of .72 that the differences observed in the mean sex ratio from litter size to litter size (for litters of 4–10 pigs inclusive) could have resulted from errors of random sampling. This indicates (as is to be expected from the mechanism of sex determination) that the sex ratio is not affected by litter size. HAINES (1931) found this true of guinea pigs also.

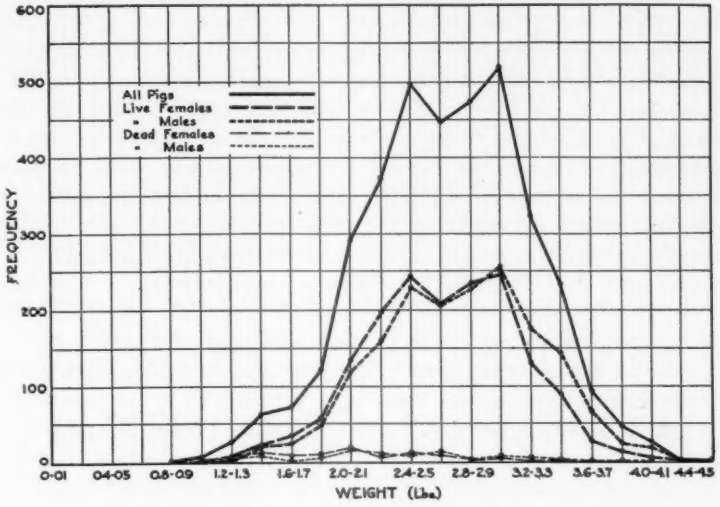

FIGURE 1.—Frequency distribution of birth weights of swine in these Iowa data.

TABLE 1

Mean birth weights of pigs.

	NUMBER OF PIGS	RANGE (LBS.)	MEAN BIRTH WEIGHT (LBS.)	STANDARD DEVIATION
All pigs	3639	0.8–4.5	2.69	.55
Live males	1755	1.0–4.5	2.78	.54
Live females	1672	1.2–4.3	2.66	.51
Dead males	106	0.8–4.0	2.31	.71
Dead females	106	1.0–3.8	2.11	.80

Effects of sex on birth weight

Data collected by CARMICHAEL and RICE (1926) for 5188 pigs show birth weights ranging from 0.2 pounds to 4.9 pounds, males generally being heavier than females. Similar observations were made by MCKENZIE (1928) and by KUHLMAN (unpublished).

The distribution of the number of pigs in each weight class in this study is shown in figure 1. The bimodality in the curves does not seem to have any biological basis. It is more likely to have resulted from the observers having an unconscious psychological bias in favor of even pounds and half pounds when weighing the pigs. The plan was to weigh the pigs accurately to the nearest tenth of a pound, but the classes including whole and half pound weights show excesses as compared with the neighboring classes. Table 1 shows the important data about birth weights according to sex and according to whether they were alive or dead at the time of birth. Males are slightly but significantly heavier than the females. Pigs born alive are heavier than pigs born dead. Pigs born dead vary more in weight than live pigs do. The analysis of variance shows that the sex difference is highly significant (P is far less than .01) yet the variance among pigs of the same sex is only 1.3 percent less than the variance in the total population of 3639 pigs. Among the pigs born alive, eliminating the sex difference reduces the variance 1.1 percent. The conclusion seems inescapable that the sex difference in birth weight is real but comparatively unimportant among the other causes of differences in birth weight. HAINES (1931) also found the sex difference in the birth weights of guinea pigs slight and was not quite certain of its significance. However he found males rather consistently heavier than females, just as we do here.

When the difference between the mean weights of those pigs born dead and those born alive is removed, the remaining variance is 4.8 percent less than the original variance, thus showing that for birth weights the difference between alive and dead is more important than the difference between male and female. It is likely that some of those born dead may have died several days before birth. Their smaller and more variable weight therefore partly results from variation in their stage of development at the time of death, as well as from whatever conditions caused their death.

Litter size and birth weight

The average birth weights for the different litter sizes are shown in table 2 and figure 2. The mean litter size for the 506 Iowa litters (all out of gilts) is $7.19 \pm .07$ pigs per litter. The birth weights reported by CARMICHAEL and RICE (1926), MCKENZIE (1928) and KUHLMAN (unpublished) are given for comparison. As a whole, the Iowa data are similar to the other data. The heavier pigs are farrowed in the smaller litters although the relation seems not to be quite rectilinear. In all four sets of data, pigs in litters of 1 or 2 are not quite as heavy as those in litters of about 3 to 5 pigs. Apparently the conditions which lead to the conception of exceedingly small litters are not the optimum for developing large pigs among

TABLE 2

Relation between average birth weight and litter size.

NUMBER IN LITTER	IOWA STATION		CARMICHAEL AND RICE		MCKENZIE		KUHLMAN		MULTIPLY ACTUAL BIRTH WEIGHTS BY THE FOLLOWING FACTORS TO CORRECT TO A STANDARD LITTER SIZE OF NINE PIGS
	NUMBER OF LITTERS	MEAN BIRTH WEIGHT (LBS.)	NUMBER OF LITTERS	MEAN BIRTH WEIGHT (LBS.)	NUMBER OF LITTERS	MEAN BIRTH WEIGHT (LBS.)	NUMBER OF LITTERS	MEAN BIRTH WEIGHT (LBS.)	
1	4	2.15	5	2.46	..	..	2	2.50	1.07
2	8	2.89	14	2.79	1	2.19	..	..	.94
3	19	2.81	19	2.82	14	2.68	4	2.67	.91
4	31	3.19	40	2.75	14	2.40	8	2.97	.90
5	57	2.87	54	2.80	8	2.67	14	2.61	.92
6	65	2.77	62	2.62	28	2.41	19	2.60	.94
7	89	2.74	80	2.65	23	2.27	32	2.60	.96
8	73	2.73	83	2.62	31	2.32	27	2.67	.98
9	81	2.60	69	2.46	36	2.41	36	2.50	1.00
10	52	2.54	81	2.53	29	2.23	35	2.53	1.02
11	18	2.53	54	2.47	29	2.35	33	2.34	1.03
12	8	2.50	39	2.45	13	2.05	19	2.47	1.05
13	1	2.13	22	2.41	7	2.28	11	2.12	1.06
14	..	..	15	2.41	7	2.34	6	2.15	1.08
15	..	..	4	2.51	1	2.98	6	2.53	1.09
16	..	..	2	2.40	..	..	4	1.91	1.10
17	..	..	..	..	1	2.26	..	..	1.11
18	..	..	1	2.38	..	..	..	..	1.12
20	..	..	1	2.51	..	..	..	..	1.12
Total	506	2.69	645	2.55	242	2.33	256	2.47	

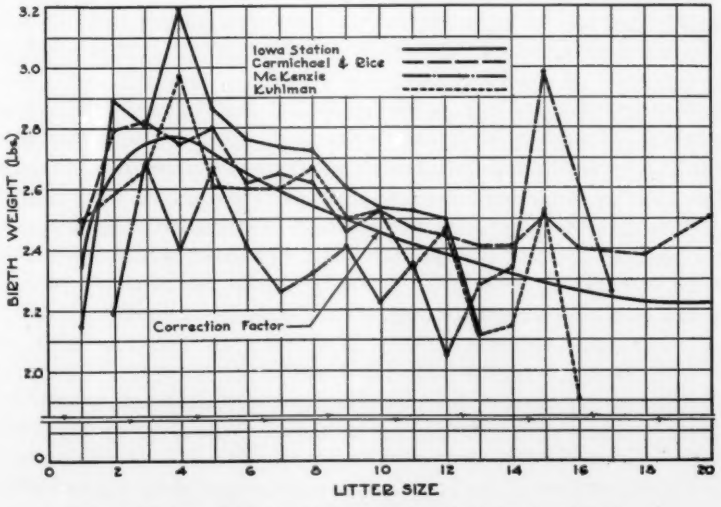

FIGURE 2.—Average birth weights for pigs born in litters of various sizes.

those which are conceived. This is somewhat similar to HAINES' (1931) finding that the lowest *mortality* in guinea pigs was in litters of two or three rather than in litters of one or in very large litters.

Of the variance for all pigs, 7.0 percent disappears when the effects of differences in litter size are removed. The corresponding figure obtained in studying only the pigs born alive is 7.7 percent. The effect of litter size is highly significant. Yet the seven percent of variance removed by excluding the effects of litter size appears small in contrast with the ninety-three percent of the original variance still remaining when attention is confined to pigs all of which were born in litters of the same size. A difference of one more or one less young in each litter has much less effect on the average birth weight in swine than in guinea pigs (HAINES) or in sheep (unpublished data of the IOWA STATION).

In the last column of table 2 are shown factors for correcting birth weights to a standard litter size of nine pigs born. These factors are based on all four sets of data with due regard for the fact that the mean birth weights in the four sets of data are not identical. The correction factors are somewhat arbitrarily adjusted to give a smooth curve with an increasing birth weight up to litters of four pigs and then a birth weight which decreases rapidly at first but somewhat more slowly with each additional pig as the litter size passes nine to eleven. The smoothing was undertaken on the hypothesis that most of the sharp irregularities observed in the actual curves are sampling errors. The numbers of extremely small and extremely large litters are so few that the correction factors for litters smaller than four or larger than twelve must be regarded as much more tentative than the others. The whole set of correction factors is presented for tentative use until extensive study of more data will justify their revision. In using these factors, the actual birth weight should be multiplied by the appropriate factor to obtain the most probable estimate of what that birth weight would have been if the pig had been born in a litter of nine, all other conditions remaining the same. For example, a pig actually weighing 3.8 pounds in a litter of four would be estimated to have weighed 3.42 pounds ($3.8 \times .90$) if it had been born in a litter of nine, while a 3.8 pound pig actually in a litter of twelve would be estimated to have weighed 3.99 if in a standard litter of nine pigs.

How litter size exerts its effect

Litter size might affect birth weight either by changing the intensity of competition among the developing fetuses for the available food supply, oxygen, space, et cetera, or by affecting the length of the gestation period so that larger litters might be born at an earlier stage of development than smaller litters. WRIGHT (1921, see page 570) found in guinea pigs that "size

of litter has much more effect on birth weight by reducing the rate of growth of the fetuses than by causing early parturition." The data necessary for a similar analysis for swine are the three correlations between birth weight (B), litter size (L), and length of gestation period (G). The other symbol (R) in figure 3 stands for the growth rate of the individual fetus. The solution is rigorously correct only if the causal relations are pictured correctly in figure 3 and if those relations are linear. The relation between litter size and birth weight is not quite linear but the discrepancy thus introduced is small. The needed correlations were computed directly (in the course of an analysis for another purpose) for the Poland Chinas

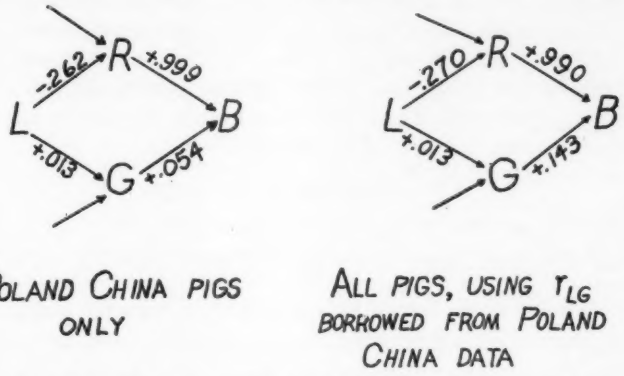

FIGURE 3.—Path coefficient diagram showing that the relation of litter size to birth weight results mostly from intra-uterine competition, rather than through intermediate effects on length of gestation.

(1261 males and 1192 females) included among these pigs. Two of those correlations may also be deduced for the larger population from the analysis of variance. These primary correlation coefficients are:

	r_{BL}	r_{BG}	r_{LG}
Computed directly for Poland China pigs	−.261	+.051	+.013
Deduced for all pigs from reduction in variance	−.265	+.141	....

Figure 3 shows the solutions when the directly computed coefficients are used and also when the deduced coefficients are used and r_{LG} is assumed to be +.013 as it was in the smaller group of data. The two solutions differ but slightly, especially with reference to the path through which litter size exerts its influence. Competition between the fetuses is indicated as the major way in which litter size affects birth weight. The effect by way of an influence on gestation length is even less important than in guinea pigs. The slight errors in assuming linearity can scarcely

have been important enough to invalidate these conclusions. Parenthetically it should be stated that gestation length is comparatively stable in swine, the mean for these 506 litters out of gilts being $115.76 \pm .06$ days with a standard deviation of 1.89 days. The extreme lengths were 107 days and 127 days.

Breed differences in birth weight

When the data are grouped according to the breed of the sire and the dam there are nine classes. Since not all breeds were equally represented in each year, it is not possible rigorously to separate breed differences from environmental factors which may have varied from year to year. The variance within breed is 3.9 percent less than the original variance in birth weights among all 3639 pigs. Among the pigs born alive the variance is reduced 6.8 percent by taking out the difference between breed means. These effects of "breed" are highly significant although not quite as important as litter size. The extent to which the effects of year possibly are included under "breed" will be discussed later.

Yearly variations in birth weight

Environmental conditions, such as weather, kind and quality of feeds, details of management, et cetera, naturally varied somewhat from season to season and may have influenced the pre-natal development of the pigs. In table 3 is shown the analysis of variance when the birth weights of the pigs are grouped according to the year in which they were farrowed. The effect of "year" is highly significant (4.714 is about 16 times .285) and yet the original variance (.302) is reduced only about 5.6 percent (to .285) when only pigs born within the same year are considered. The corresponding reduction among the pigs born alive is 6.4 percent.

TABLE 3

Analysis of variance in birth weights by years.

VARIANCE DUE TO	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	STANDARD DEVIATION
"Year"	14	66.00	4.714	
Other causes	3624	1032.00	.285	.534
All causes	3638	1098.00	.302	.549

Combined effects of year and breed

When year and breed are treated separately each is found to be associated significantly with variations in birth weight. If each breed had constituted a constant proportion of the pig crop in all years, the combined importance of breed and year could have been obtained by adding the separate figures together (possibly plus a small amount for genuine dif-

ferences in the way different breeds reacted to differences in the environment from year to year). Since they were not so distributed it is *a priori* likely that each, figured separately, contains some of the effects of the other and that a simple addition of their separate effects would result in an exaggerated estimate of their combined importance. Actually only 7.6 percent and 11.4 percent of the original variance for all pigs and for live pigs respectively disappears when the pigs are divided into groups within each of which all pigs belonged to the same breed and were born in the same year. These figures, while larger than for either "breed" or "year" alone, are smaller than the sums (9.5 percent and 13.2 percent) of their effects when figured separately. It is thus clear that "breed" and "year" when figured separately each include some effects of the other. The figures for "year and breed" (7.6 percent and 11.4 percent) express their combined importance but it does not seem possible to estimate very closely from these data the separate importance of each.

Does the sire influence the birth weight of his pigs?

Litter-mates are subject to similar environmental conditions during their intra-uterine life. This might well make the dam more important than the sire in affecting birth weights, even though both sire and dam contribute equally to the genes of the embryo. To find whether the boar had any effect at all upon the birth weight of his pigs, all pigs born in the same year and sired by the same boar were grouped together. It was found that 6.2 percent and 7.0 percent of the variance for all pigs and for those born alive was eliminated when this was done. These figures are to be compared with those for "year" alone (5.6 percent and 6.4 percent). Even though the variance eliminated by holding sire constant within "year" is but slightly more than that eliminated by "year" alone, P is about .01 and it appears certain that the boar had some effect on the birth weights of his offspring. Table 4 shows the data which support this conclusion.

TABLE 4

Variance by years and by sires.

VARIANCE DUE TO	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
"Year"	14	66.00	4.714
Boar within year	18	10.29	.572
Remainder	3606	1021.71	.283
Total	3638	1098.00	.302

The additional portion of the variance which disappears when sire as well as year is "held constant" provides a basis for estimating the cor-

relation between sire's genotype (that is, the sum of the average effects of the genes possessed by him) and pig's birth weight. Thus $\sqrt{.062-.056}$ gives .08 as an estimate of the correlation between paternal genotype and offspring's birth weight. The difference between .062 and .056 when multiplied by four leads to the estimate that between two and three percent of the variance in birth weight is due to differences in the genes possessed by the pigs themselves. This estimate must be regarded as quite provisional for the following reasons:

1. The indirect method of its computation provides opportunity for sampling errors to be magnified, thus making it possibly in error in either direction.
2. If part of the difference between years is really genetic (as may well have been the case), this estimate is too low.
3. Variance due solely to dominance or to complex non-cumulative interaction of genes is not included in this estimate.
4. Assortative mating within years (if that occurred) would have made this estimate too high.

While the estimate that in these data a little less than 3 percent of the variance in birth weight is due to differences in the genes possessed by the pigs themselves must therefore be considered highly tentative, it seems as reasonable an estimate as can be made at present.

In crossbreeding experiments between highly inbred strains of guinea pigs, WRIGHT (1922) finds that: "Birth weight depends largely on the dam — about three-fourths — and only one-fourth on the breeding of the young." His statement here concerns the average birth weights of all pigs from each cross and does not include the individual variation from pig to pig which is so prominent in the variance discussed in the preceding paragraph.

GREGORY and CASTLE (1931) state that embryonic development in the rabbit proceeds at a more rapid rate in rabbits of large races than in rabbits of small races. The higher rate of development of these rabbits is influenced by the sperm, as well as by the egg and may be supposed to be induced by chromosomal genes. They used races selected expressly because of a wide contrast in size and hence would be expected to find a larger proportion of genetic differences than in these swine which were selected with no conscious attention to birth weight and without any attempts to secure extreme contrasts in adult size.

Effects of differences between reasonably good rations

To determine whether differences in the moderately good rations fed to these sows had any effect on the birth weights of their pigs, the birth weights within each year were grouped according to the ration the dams

received. Table 5 shows the variance between pigs born in the same year but classified according to the ration of their dams. Year and lot together account for 9.6 percent and 10.3 percent of the variance in birth weight for all pigs, and for pigs born alive. The effect of ration is relatively small, yet statistically quite significant. The rations were all reasonably good ones. Had extreme rations, such as corn and water alone in dry lots, been included, no doubt a more extreme effect of ration would have been found.

Length of gestation period

According to CARMICHAEL and RICE (1926) the length of the gestation period does not appear to have any appreciable effect on the birth weights of the pigs. However they found a slight tendency for some of the litters which were carried longer than the average to have fewer pigs than those that were farrowed earlier. This observation suggests that the effect of gestation length on birth weights is involved with the effect of variation in litter size, as is discussed earlier in this paper under the discussion of litter size. In the present study when gestation period was "held constant" about 2.0 percent of the variance in birth weights was decreased for all pigs and 3.5 percent for live pigs. This effect is statistically significant, although not very important.

TABLE 5

Analysis of variance in birth weights by lots.

VARIANCE DUE TO	DEGREES OF FREEDOM	SUM OF SQUARES	STANDARD DEVIATION
"Year"	14	66.00	4.714
Lot within year	52	55.30	1.064
Other causes	3572	976.70	.273
All causes	3638	1098.00	.302

"Condition" and "vigor" of the pigs

Condition and vigor of the pigs were graded at the time of birth. These terms designate certain characteristics of the pigs, such as the state of health, the general conformation and the apparent vitality and strength of the pigs. The precision with which the animals were classified naturally is affected by the personal opinion of the observer in spite of all precautions against this. Condition and vigor can in no sense be regarded as *causes* of the differences in birth weights. They are in large part additional ways of expressing the same things which are expressed in birth weights. The analysis of variance shows roughly how much of what the caretaker saw when he was looking for differences in condition and vigor was also reflected in differences in weights. The variance in birth weights within

groups of pigs given the same grade for condition is 50.7 percent less for all pigs and 37.5 percent less for pigs born alive than that found within the whole group of observations. Similarly 38.4 percent of the mean variance for all pigs (38.8 percent for pigs born alive) is eliminated when the data are grouped according to the grade for vigor.

Weight differences between litter-mates

Litter-mates are more apt to have the same genes than are pigs less closely related than full brothers and sisters. Yet litter-mates are unlike in many genetic factors. Litter-mates also tend to be alike because they developed in the same uterus and were thus exposed to an environment remarkably uniform for members of the same litter but perhaps differing distinctly from litter to litter. Yet there must have been environmental differences from one part of the uterus to another, as perhaps in the crowding or in the blood supply to each fetus. A comparison of the variance between litter-mates with the variance between all pigs, permits a rough estimate of the importance of intra-uterine environment which differs from litter to litter plus genetic differences between litters as contrasted with genetic differences within litters plus "accidents of development"—that is to say, environment which affected differently even pigs in the same uterus.

In a random-bred population the genetic variance between litter-mates would be about half as large as the genetic variance of individuals chosen at random from the whole population. However this stock was not quite random-bred since several breeds and crosses were included. One would expect the genetic variance between litter-mates to be slightly less than half as large as the genetic variance in the entire population, if these breeds and crosses differed genuinely from each other in birth weights. The group studied corresponds crudely to a population of several very slightly inbred lines (unselected for birth weights) with a few crosses between those lines.

The variance in birth weight of litter-mates is 50.7 percent less than that in the whole population. The corresponding figure for live pigs only is 54.6 percent. The portion of the total variance thus eliminated includes that due to all controlled environmental conditions (that is, all effects of environment which were identical for litter-mates) and also probably a little more than half of the genetic differences. The remaining variance (49.3 and 45.4 percent) is that due to environment which differed even among litter-mates and to genetic differences between litter-mates, also including sex.

Using the data for all pigs and subtracting from the 49.3 percent of the variance between litter-mates about half of the genetic portion of the

variance already mentioned in discussing the influence of the sire, and also subtracting the variance due to sex (1.3 percent) there is left about 47 percent of the original variance due to environment not common to litter-mates. Similarly, subtracting from the 50.7 percent of the original variance not found between litter-mates a little more than half of the genetic variance within breeds and the variance between breeds within year (2 percent), there is left about 47 percent of the original variance due to environment which differed from litter to litter but affected all litter-mates alike. This yields the following rough apportionment of the variance due to various causes in these data:

Heredity of the pigs	*Percent*	
Breed differences	2	
Sex	1	
General	3	
		6
Environment common to litter-mates		
Litter size	7	
Year	5	
Ration	4	
Gestation length	2	
Other	29	
		47
Environment not common to litter-mates		
		47
	Total = 100	

The following reservations should be kept in mind in interpreting this apportionment. First, if pig birth weight is largely the expression of genetic differences between dams in somewhat the same way that egg size in poultry is largely the expression of maternal differences, regardless of the kind of sperm which fertilized the ovum, then some of the "Other" environment common to litter-mates is really genetic in origin but depends on the genotype of the dam rather than on the genotype of the pig. WRIGHT (1922) found this to be the case in crosses of inbred lines of guinea pigs. Second, random errors in weighing (as were mentioned in discussing the apparent bimodality of the principal curve in figure 1) would appear as part of the environment not common to litter-mates. Third, differences in weight because some litters had suckled and others had not, would appear as part of the "Other" environment common to litter-mates.

Fourth, variance due to dominance or to non-cumulative interaction of the genes would appear both under environment common to litter-mates and under that not common to litter-mates but most of it would be under the latter. The total amount of variance from dominance or non-cumulative interactions can hardly have been large, however, since the total part played by the heredity of the pigs appears to have been small. Fifth, these proportions apply to these particular data which include a wide sample of rations and years and several breeds but only litters produced by gilts. Had the breeds been selected expressly to secure a wide range in size (as in experiments such as those of GREGORY and CASTLE) the breed differences would no doubt have appeared proportionately larger. Had litters from older sows been included, there would have been an item under "environment common to litter-mates" for age of dam and all the other items would have been reduced somewhat in proportion. Had rations been more extreme in their contrast, ration would have been relatively more important and the others less so. Sixth, a portion of the variance ascribed to "year" may really have been genetic, since none of the dams were used in two years and few of the boars were used more than two years.

For these reasons, and because of the indirect methods by which it was computed, this apportionment must be considered provisional and not securely enough founded to justify any broad generalizations except perhaps the one that the genetic portion of the variance in birth weights within a breed of swine is comparatively small. In spite of these reservations this apportionment seems to provide as complete and reasonable a picture of the general situation as the present data and state of knowledge afford.

SUMMARY AND CONCLUSIONS

1. Birth weights of 3639 pigs in 506 litters farrowed by gilts at the IOWA AGRICULTURAL EXPERIMENT STATION were studied to find the importance of various sources of variation in those weights.

2. The sex ratio of the pigs at birth was 51.1 percent males. The frequency of the sex combinations seemed to be a random expression of $(p+q)^n$. The sex ratio was not related to litter size. Males were significantly but very slightly heavier than females.

3. The mean litter size for all breeds was $7.19 \pm .07$ pigs. The relation between litter size and birth weight is slightly curvilinear, the maximum birth weights being in litters of about 3 to 5 pigs each. Tentative factors are suggested for correcting birth weight to a standard litter size of nine pigs born.

4. The following portions of the variance in individual birth weights disappeared when each of the named variables in turn was held constant but the others were allowed to vary:

VARIABLE	PORTION OF VARIANCE IN PERCENT WHICH DISAPPEARED	
	AMONG ALL PIGS	AMONG LIVE PIGS ONLY
Sex	1.3	1.1
Alive at birth	4.8	..
Litter size	7.0	7.7
"Breed" alone	3.9	6.8
"Year" alone	5.6	6.4
"Year" and "breed" together	7.6	11.4
Year and sire together	6.2	7.0
Year and ration together	9.6	10.3
Length of gestation	2.0	3.5
"Condition" of pig at birth	50.0	37.5
"Vigor" of pig at birth	38.4	38.8
Litter	50.7	54.6

Not all of these variables are independent of each other but many overlap considerably. Hence (and because not all causes of variation are included) these percentages do not add up to 100 percent.

5. A rough approximation is given of the relative importance of heredity and environment as causes of variation in birth weight in these data.

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THE RELATION OF A DOMINANT EYE COLOR IN DROSOPHILA MELANOGASTER TO THE ASSOCIATED CHROMOSOME REARRANGEMENT

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The possibility that the developmental effects of genes are influenced by their neighbors in the chromosomes has only recently become accessible to experiment. The first demonstration of such an effect was given by STURTEVANT (1925). Two Bar genes in *Drosophila melanogaster*, placed in the same chromosome as a result of unequal crossing over, are more effective than two similar genes present in the two chromosomes of a homozygote. The expression of the Bar gene is dependent upon its position in the chromosome.

It was early evident (STURTEVANT 1925, p. 138) that chromosome rearrangements should provide further data for the statement and solution of the problem. Following a chromosome rearrangement, the genes adjoining the break change neighbors. If the behavior of a gene is a function of its relations to its neighbors, at the points of breakage in chromosome rearrangements, changes resembling mutations should be found. In point of fact, the original "Pale-translocation" was detected by virtue of a change inseparable from the locus of the translocation (BRIDGES 1923, BRIDGES and MORGAN 1923).

With the advent of the X-ray technique for producing chromosome rearrangements on a large scale, the problem was really opened to attack. In the X-ray translocations, MULLER and ALTENBURG (1930) and DOBZHANSKY (1930) have shown that most translocations in *Drosophila melanogaster* contain lethal "mutations" at the point of fracture. And, as is usual in the relation between lethal and non-lethal mutants, where many of the former are found, some of the latter also occur.

A few cases involving known mutants at the breaks have been studied in detail. DOBZHANSKY (1932) analyzed a translocation in which the X chromosome was broken at the Bar locus, the second chromosome at vestigial, and an interchange effected. At the point of translocation, in place of the wild-type section present in the treated chromosome, a new allele of Bar was found. The second mutation from wild-type to Bar, in all the

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intensive work done on Drosophila, occurred at the locus of a chromosome rearrangement. STURTEVANT (1928) has already shown that there is no effective wild-type allele of Bar. The "baroid" mutation can therefore not be a simple deficiency, caused by a loss of material due to irregularity of union of the broken pieces. It is, however, possible to understand these data on the assumption of a position effect similar to that which STURTEVANT had shown to be operative in the case of Bar itself.

This assumption is also useful in another series of cases, involving the bobbed locus in the X chromosome. STERN (1931) had noted that in several spontaneous translocations involving X and Y, when a break occurred at the bobbed locus, a mutation to bobbed was found. SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933) found in a whole series of duplications for the X chromosome, where one break had occurred in the inert region, that a correlated mutation to bobbed was present.

Yet, as was pointed out in the case of baroid and of the duplications for the X chromosome, although the position effect hypothesis provides a sufficient explanation of the data, there are other possibilities, which might be advanced for individual cases. For example, at breakage points, mutations might be more likely to occur (MULLER and ALTENBURG); or there might be actual losses of genic material during the rearrangement, as BRIDGES supposed for the case of the Pale-translocation. The problem is to distinguish between these alternatives.

The dominant eye colors induced by X-ray treatment in *Drosophila melanogaster*, furnish excellent material for such an analysis. A series of these have been reported by WEINSTEIN (1928), MULLER (1930), VAN ATTA (1932), and GLASS (1932). Most of them belong to the class of "ever-sporting" mutants, showing a distinct variegation in the eye color. The significant point for our present purposes is MULLER'S observation, upheld by later work, that these dominant eye colors are correlated with chromosome rearrangements.

In this paper, we report a new member of this series, together with a comparative analysis of its behavior and that of another member of the series. From the study of the chromosomal structure of our mutant, we have determined the loci of the breakage points. These determinations we have correlated with a study of the allelism of the dominant effect to known recessive eye colors located at these breakage points. The results of this analysis are precisely those to be expected on the position effect hypothesis, and we have been able in this case to exclude the possibility that only a deficiency at the break may account for these effects.

It is a pleasure to thank Dr. C. B. BRIDGES for his generous permission to use unpublished data. We are also indebted to Miss MILDRED GROSCURTH for assistance in some of the experiments.

THE ORIGIN OF PLUM-2, AND ITS ALLELISM TO PLUM-1

Plum-2 occurred as a single female, in the progeny of an X-ray experiment. Freshly hatched Bar males had been treated with X-rays (50 KV, 5 ma, 15 cm, 60 minutes, 0.8 mm Al filter), and mated to females containing attached X chromosomes homozygous for the recessives vermilion (*v*), sable (*s*), garnet-2 (g^2) and forked (*f*). Among about forty-two thousand offspring of this cross ($v\ s\ g^2\ f$ ♀ ♀, *B* ♂ ♂), one female showed a pale lemon-yellow eye color with numerous ill-defined spots of a darker yellow, instead of the light orange of the vermilion garnet-2 combination (culture No. 27765, July 6, 1932).

This female was mated to an unrelated wild-type male, and in F_1 the mutant eye color appeared in both males and females (table 1).

TABLE 1

	$v\ s\ g^2\ f\ \widehat{XX}\ Pm^2/+$ ♀ × + ♂		
$v\ s\ g^2\ f$ ♀	+ ♂	$v\ s\ g^2\ f\ Pm^2$ ♀	Pm^2 ♂
8	12	6	9

This showed that an autosomal dominant was present. Freed of vermilion garnet-2, the new mutant resembled Plum-1, described by MULLER (1930). The ground color of the eye is brownish, with deeper purplish patches, the general hue then appearing as a brownish purple. The interaction with vermilion garnet-2 produced the pale yellow of the original female and her daughters.

By the usual linkage tests it became apparent that no translocation was present, and that Plum-2 was in the second chromosome. The cross of Plum-2 to the standard second chromosome balancer, the dominant mutant Curly wing with its associated inverted sections permitted a stock of the constitution Plum-2/Curly to be established. In this stock only the heterozygotes appear. Curly is known to be lethal when homozygous in stock cultures, and the failure of the Plum-2 homozygotes to survive is due to a recessive lethal effect in the Plum-2 chromosome.

This behavior of Plum-2, as well as its appearance, was so similar to MULLER's Plum-1 that a cross was made between the two balanced stocks, Plum-1/Curly and Plum-2/Curly. Only Plum Curly flies appeared in F_1, indicating that Plum-1/Plum-2 is a lethal combination. Since there is no likelihood of the presence of any miscellaneous lethal common to both chromosomes, this demonstrates the allelism of Plum-1 and Plum-2.

PLUM-2 ASSOCIATED WITH AN INVERSION IN THE RIGHT LIMB OF CHROMOSOME II

Three different sets of experiments were carried out, which sufficed to show in some detail what changes from the normal second chromosome are present in Plum-2.

In the first experiment, it became apparent that Plum-2 involves an inversion in the second chromosome. Crossing over was studied in heterozygous females of the composition Plum-2 (*Pm²*)/aristaless (*al*) Bristle (*Bl*) curved (*c*) speck (*sp*) (table 2). In this way, a survey of the crossing

TABLE 2

	Pm²?		*Pm²*?	♀♀ × *al c sp* ♂♂	
	al (1) *Bl* (2)	(3) *c* (4)	(5) *sp*		
0	*Pm²*	1258	5	*al Bl c*	1
0	*al Bl c sp*	1240	1, 2	*al c sp*	1
1	*al Pm²*	955	3, 4	*al Bl sp*	10
1	*Bl c sp*	1058		*Pm² c*	11
2	*al Bl Pm²*	1	1, 3, 4	*al Pm² c*	2
2	*c sp*	1	1, 3, 4	*Bl sp*	1

TOTAL 4539

over throughout the chromosome was obtained. The results of the cross appear in table 2. It is clear at a glance that crossing over in the right limb of the chromosome is markedly suppressed. The percentages for the various intervals follow:

al-Bl	*Bl-Pm²*	*Pm²-c*	*c-Pm²*	*Pm²-sp*
44.43	0.07	0.53	0.53	0.02

These are to be compared with map values:

al-Bl	*Bl-c*	*c-sp*
54.7	20.8	31.5

The discrepancy with the map value in the left limb is due to undetected double crossing over. In the right limb, however, not only is the total amount of crossing over reduced, but the crossovers occurring in the mid-portion are all doubles. Between Plum-2 and speck, single crossovers are again found. The data may be explained simply on the basis of an inverted section of the right limb of the second chromosome, not including the end. Within an inverted section, only double crossovers are found; outside it single crossovers occur, but with reduced frequency (see STURTEVANT 1931). From this interpretation, it follows that Plum-2 must have its locus close to the ends of the inversion, since none of the rather frequent doubles between the inversion and the normal chromosome involved Plum-2.

The second experiment served to confirm this analysis. A more completely marked chromosome was used opposite the Plum-2 inversion. Crossing over was studied in females heterozygous for Bristle Plum-2, and the multiple stock carrying the recessives aristaless (*al*), dumpy (*dp*), black (*b*), purple (*pr*) curved (*c*), plexus (*px*), and speck (*sp*). These data are shown in table 3, and the computed percentages in table 4. Plum-2 and

purple, eye colors which on occasion are difficult to distinguish, were disregarded in these counts.

TABLE 3

(1)	(2)	(3)	(4)	(5)	(6)				
al	*dp*	*b*	*pr*	*c*	*px*	*sp*	♀♀ × *al dp b pr c px sp* ♂♂		
			Bl	*Pm*²?		*Pm*²?			
				(*Pm*² and *pr* disregarded)					
0		*Bl*		396		1, 3	*dp b Bl*		6
0		*al dp b pr c px sp*		358		1, 3	*al c px sp*		6
1		*al Bl*		88		2, 3	*b Bl*		8
1		*dp b c px sp*		77		2, 3	*al dp c px sp*		7
2		*al dp Bl*		194		4, 5	*Bl c*		3
2		*b c px sp*		244		4, 5	*al dp b bx sp*		3
3		*al dp b Bl*		49		4, 6	*Bl c px*		2
3		*c px sp*		47		4, 6	*al dp b sp*		2
6		*al dp b c px*		1		1, 4, 5	*dp b px sp*		1
6		*Bl sp*		1		2, 4, 5	*b px sp*		2
1, 2		*dp Bl*		5		2, 4, 5	*al dp Bl c*		2
1, 2		*al b c px sp*		8					
				TOTAL 1510					

TABLE 4

Crossover values computed from the data of table 3, compared with the map values.

	Data of Table 3	Map Value (BRIDGES)	Difference
al-dp	12.7	13.0	− 0.3
dp-b	31.1	35.5	− 4.4
b-Bl	8.2	6.5	+ 1.7
Bl-c	1.0	20.5	−19.5
c-px	0.7	25.0	−24.3
px-sp	0.4	6.5	− 6.1

As in the first experiment, the values for the left limb are near normal; and in the right limb, crossing over is suppressed. From these data, a further estimate may be made of the region involved in the inverted section, and hence of the loci of the points of breakage in the chromosome. Single crossovers occur between Bristle and Plum-2, giving a point not far to the right of Bristle as the locus of one of the breaks. Only double crossovers are found involving curved and plexus, which implies that these genes lie within the inverted section. Single crossovers between plexus and speck are again found, indicating that the locus of the second break is between plexus and speck. Four double crossovers involving the region between plexus and the end of the inversion were found in 1510 flies; two single crossovers occurred in this experiment between the end of the inversion and speck. It appears that the distal end of the inversion is closer to speck than to plexus. The locus of the right break in the chromosome is two-thirds of the distance from plexus to speck, or at the locus of brown (104.5) in the second chromosome.

A third experiment was carried out to locate the proximal end more exactly. Crossing over was studied in females heterozygous for Bristle Plum-2 and cinnabar (*cn*) whose locus is at 57.5, 2.8 units to the right of Bristle. The results are shown in table 5.

TABLE 5

$$\frac{Bl \quad Pm^2}{(1) \quad (2)\ cn} \text{♀ ♀} \times cn \text{♂ ♂}$$

(0)		(1)		(2)		
Bl Pm²	*cn*	*Bl cn*	*Pm*	*Bl Pm cn*	+	TOTAL
2458	2675	4	10	8	8	5163

The crossovers between Plum-2 and cinnabar engage our attention. If cinnabar lies within the inverted section, these are all double crossovers. Then the *Bl Pm² cn* flies carry the inversion, and the contrary crossover class (wild-type) is free of it. Conversely, if cinnabar lies to the left of the inversion, these are single crossovers, and the wild-type flies carry the inversion, the cinnabar flies do not. Tests were made of four crossovers of each type, in which the crossing over was observed in heterozygotes for *al dp b pr c px sp*. It was found that the *Bl Pm² cn* chromosomes carried the inverted section, and the wild-type chromosome did not. The proximal break in the inverted section lies therefore between Bristle and cinnabar, close to the spindle attachment.

Since the proximal break of the $C_{IIR}\ c_v$ inversion is close to cinnabar (GRAUBARD 1932) it seemed desirable to make a test of crossing over in the heterozygote for Plum-2 and Curly. If the inverted sections in the two chromosomes correspond, crossing over should increase from the low value observed between either inverted section and the normal chromosome, to an approximately normal amount (STURTEVANT 1931, GRAUBARD 1932). Crossing over was therefore studied in females of the constitution aristaless Curly $C_{IIL}\ c_v\ C_{IIR}\ c_v$ Lobe^c speck/Plum-2 curved. The data are presented in table 6.

TABLE 6

$$\frac{al \quad Cy\ C_{IIL}\ c_v \quad C_{IIR}\ c_v\ L^c \qquad\qquad sp}{(1) \quad (2)\ Pm^2?\ (3) \quad (4)\ c\ (5)\ Pm^2?\ (6)} \text{♀ ♀} \times al\ b\ c\ sp \text{♂ ♂}$$

0	*Pm c*	2041	3, 4	*al Cy sp*	4
0	*al Cy L^c sp*	1933	3, 5	*al Cy c sp*	4
2	*al Cy Pm² c*	16	3, 5	*Pm² L^c*	5
2	*L sp*	14	4, 5	*al Cy L^c c sp*	2
6	*Pm² c sp*	1	4, 5	*Pm²*	3
3, 4	*Pm² L c*	1	2, 4, 5	*L c sp*	2

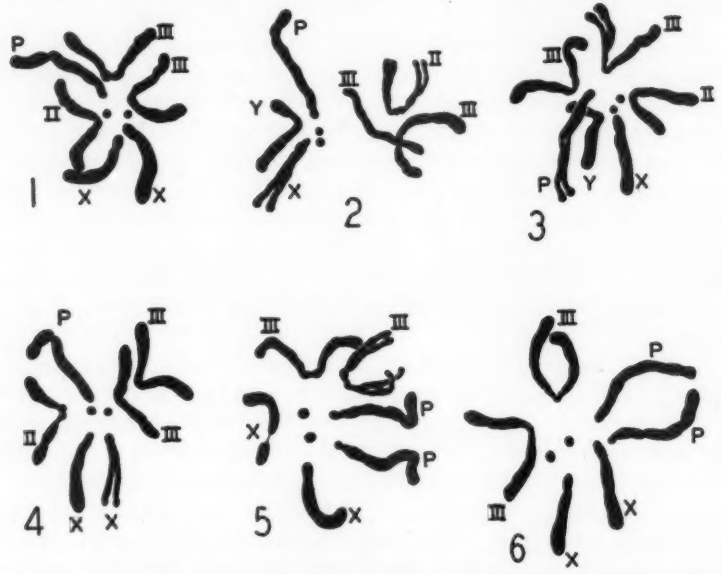
TOTAL 4026

From these data it appears that the two inverted sections do not correspond. The crossover percentages are of the same order of magnitude as

those observed for the right limb in heterozygotes for Plum and a normal chromosome. They are, from the data in table 6:

al-Cy	*Cy-Pm²*	*Pm²-L^c*	*L^c-c*	*c-Pm²*	*Pm²-sp*
0	0.79	0.34	0.29	0.17	0.02

The total crossing over is 1.61 percent, which is to be compared with a total of 2.1 percent for the right limb in table 4, and 0.50 percent in heterozygotes for the Curly and a normal chromosome (GRAUBARD 1932). The value is intermediate between those observed with the respective heterozygotes for the normal chromosomes.



FIGURES 1–6.—The chromosomes of Plum-2. Metaphase plates of nerve cells. Figures 1–4, heterozygotes; 5–6, homozygotes. The Plum-2 chromosome is designated by the letter "P"; II is the normal second chromosome; III, the third; and X and Y respectively the X and Y chromosomes. Magnification about 6500×.

THE CYTOLOGICAL DETERMINATION OF THE PLUM-2 INVERSION

The genetic data given in the previous section admit of several possible interpretations, equally compatible with the necessary assumption of two breakage points, one between Bristle and cinnabar, the other around the locus of brown. To decide between these, a cytological study was made. The nerve ganglia of full grown larvae from the balanced stock Plum-2 /*Cy* C_{IIL} C_{IIR} were fixed in Navashin's fluid, and paraffin sections prepared which were stained in gentian violet by the usual technique. Only

two types of full grown larvae appear in this stock—Plum-2/*Cy*, and homozygous Plum-2. The homozygotes for Curly usually die in the mid-larval stages, according to the data of SIVERTZEV-DOBZHANSKY (1927).

Figures 1–4 show the heterozygotes, Plum-2/*Cy*. These have one second chromosome with a median spindle fiber attachment. The other is a long rod-shaped chromosome, which must be the Plum-2 chromosome. In figures 5 and 6 the homozygous Plum-2 chromosome plates are shown. These have two of the long rods, and no second chromosome with a me-

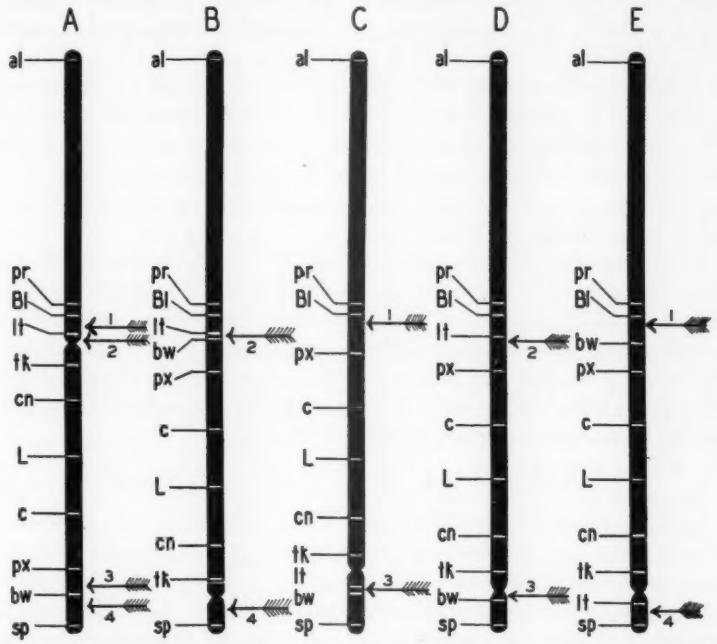


FIGURE 7.—*A*, the structure of the normal second chromosome. *B*, *C*, *D*, and *E*, the various possibilities for the chromosome structure of Plum-2. The spindle attachment is indicated by a constriction, the loci of breakage by arrows.

dian attachment. In the rod-shaped chromosome, the spindle attachment is sub-terminal. The constriction at this place is seen plainly in figure 2, in one of the chromosomes of figure 6 and in both in figure 5. It follows that the break between Bristle and cinnabar occurred to the left of the spindle attachment. Since crossing over in the left limb of the Plum-2 chromosome is normal, this chromosome must have one of the structures represented in figure 7.

A comparison of the topography of the Plum-2 chromosome with that of

the dominant eye colors previously studied presents some points of interest. The "Dilute" series described by VAN ATTA (1932) is very probably homologous with the series of alleles of Plum-1 described by GLASS (1932 and in the press). In these two series, there are four mutants which are associated with inversions. Plum-1 contains two inversions, and Van ATTA's Dilutes 1 and 2 may be interpreted similarly. The remaining member of GLASS's series has not as yet been fully described; the brief account available implies that it contains, as does Plum-2, only one inversion. This gives three out of five cases, all selected on the basis of the eye color alone, in which a double inversion has occurred. It would seem then that a correlation may exist between the formation of an inversion in one limb of chromosome II and in the other. The data are not sufficient at present to be decisive, but it should be remembered that STURTEVANT (1931) has noted a correlation of this kind in the inversions of spontaneous origin which he has studied. The data on the Plum inversions support his interpretation, that the correlation is concerned with the simultaneous origin of the double inversions.

For our present purposes, the most important point is that in all these cases, the dominant eye color is inseparable from the inversion in the right limb; which means that it has its locus at the breakage points (either or both). The rearrangement of chromosomal material at a specific locus is correlated with the appearance of a "mutation" at that locus. It is possible, by a closer analysis of the properties of the "mutation," to obtain a certain measure of information as to its nature.

CHANGES AT THE RIGHT BREAK OF THE INVERSION

It will be recalled that in the case of Plum-2, the right break was shown to have taken place between plexus and speck, at the locus of brown. The work on Plum-1 and its alleles had sufficiently established the loci involved in the changes in this region, for those cases. BRIDGES discovered the suppression of Plum-1 by a duplicating section for the plexus-speck interval (MORGAN, BRIDGES and SCHULTZ 1930). This was extended to the other members of the series by VAN ATTA (1932) and by GLASS (1932) who obtained similar suppressions in all cases. GLASS has added the significant fact that his mutants are all allelic to the recessive eye color brown, located in this region at 104.5.

Further information comes from unpublished work of Dr. BRIDGES. He has made a systematic test of Plum-1 for deficiency of the other mutants available in the plexus-speck region. The heterozygotes with Plum-1 are normal in all cases except those of brown, minus, and abbreviated. The two latter are recessive bristle characters, located respectively at 104.5, and 105.0. Their heterozygotes with Plum-1 show frequently small patches

—mainly single bristles—manifesting the mutant character. This represents a variegation similar to that observed in the cases of mottled eye reported at the white locus (MULLER 1930, PATTERSON 1932). It would appear that in Plum-1 a section of chromosome is involved in the variegation; however, further study of the problem is necessary. BRIDGES has also shown that the lethal effect of homozygous Plum-1 lies outside the plexus-speck region; the homozygote does not survive in the presence of the duplicating segment for this region. In its essentials, the above might serve as a description of the behavior of Plum-2 in these respects.

For the suppression of Plum-2, the duplicating fragment of BRIDGES' "Pale-translocation" was utilized. Crosses of the stock Pale deficiency/Curly Pale duplication/Stubble $C_{IIIR\ Mo}$ with the Plum-2/Curly stock gave individuals of the constitution Plum-2/Curly, Pale duplication. These possess in triplicate the plexus-speck region. They are all, like Plum-1 and its fellows, almost wild-type in appearance, instead of the mottled brownish color of Plum-2. Subsequently, a stock of Plum-2/Curly Stubble $C_{IIIR\ Mo}$/Pale duplication was established by Dr. BRIDGES. As he points out, the suppression of Plum-2 is even more complete than that of Plum-1. The remaining brown facets of the suppressed Plum-1 fly are rarely to be found in the case of Plum-2. The eye is usually completely wild-type. It is clear that the dominant effect is a function of some locus in the plexus-speck region.

The tests with mutants in this region make it highly probable that this locus is brown. The compounds of Plum-2 with brown show an almost homogeneous brown eye color, which darkens in older flies until an ill-defined mottling is discernible. Plum-2, like Plum-1 and the other members of the group, shows in combination with brown, the disproportionate modification characteristic of alleles. There is in addition, another criterion than eye color available in the case of brown. The testis sheath, instead of being bright yellow, as it is in the wild-type, is completely transparent in flies homozygous for this mutant gene. Plum-2 (and Plum-1 as well) has the normal testis color, in the heterozygotes for a wild-type chromosome. The compounds with brown show in all cases a variegated testis, with patches of yellow and white. There is evidently a frequent "mutation" at the brown locus; the mosaic character of the testis sheath confirms the evidence from the eye color relations in a striking fashion.

In the tests with the other mutants in this region (minus, abbreviated, speck) definite results were obtained for Plum-2 only with minus. Heterozygotes for Plum-2 and minus frequently show patches of minus bristles. These are very short stumps, and so are clearly distinguishable from the wild-type. The results in this case are completely parallel to those of Plum-1. In the case of abbreviated, which BRIDGES found to be involved

in the mosaicism in Plum-1, our evidence is not clear. No certain mosaic patches were found in several hundred individuals examined. But abbreviated is occasionally capricious in its manifestation, and in spite of the fact that the tests were made with two different stocks, further study is necessary.

The lethal effect in homozygous Plum-2, like that in Plum-1, was found to lie outside the plexus-speck region. In the case of Plum-1, this is by no means surprising, since two inversions (four breakage points) are concerned, and there may even be more than one lethal. The situation with Plum-2 is somewhat different. The homozygotes survive to a late stage—in certain stocks occasional individuals even hatch—and thus, since only one inversion is involved, it might be supposed that were any recessive locus in the brown region concerned with the lethal effect, a duplicating section for that region should suppress it and permit survival. The experiment performed was as follows: Plum-2/Curly, Pale duplication/Stubble $C_{IIIR\ Mo}$ females were crossed to Plum-2/Curly males. The cross gave:

Pm Cy Sb	*Cy*	Crossovers	Homozygous Curly classes
261	234	30	44

The homozygous Plum individuals should appear in the not Curly classes. These are completely absent, both for the Stubble classes, which do not contain the duplication, and the opposite classes which do. It follows that the lethal effect in Plum-2 lies, as previously stated, outside the plexus-speck region.

PLUM NOT A SIMPLE DEFICIENCY FOR BROWN

The situation may then be epitomized thus: the occurrence of a dominant effect is associated with a breakage at the locus of brown. This breakage is also associated with instability at the brown locus and at neighboring loci. The first statement holds for Plum-2, for all the mutants studied by GLASS, and probably for VAN ATTA'S Dilutes. The second can only be stated definitely for Plum-1 and Plum-2.

The question may be asked, cannot these data be explained on the basis of an unstable chromosome rearrangement of the type described by PATTERSON and PAINTER? The mutant portions would then be interpreted as deficient for the normal alleles of the loci involved. But it does not seem obvious at the moment how the dominant effect can be placed in such a category. Consider the flies of constitution Plum/+ Pale duplication/+. These still show brown patches, although small ones. Now were this effect due simply to a deficiency, such brown patches should not appear, since the normal complement of alleles is present at the brown locus.

Further evidence comes from studies of the behavior of Plum-1 in triploids. Wild-type triploids were crossed to males heterozygous for aristaless dachs Bristle curved speck/dumpy Plum-1. The results were:

2N		3N		Intersexes	
Bl	*Pm*	*Bl*	supp. *Pm*	+	*Bl*
68	59	6	12	43	32

The triploids of constitution Plum-1/+/+, like the diploids with the duplication, have small brown patches in an eye with a red ground color.

In the triploid there is available for comparison evidence as to the eye color of a known brown deficiency. The deficiency corresponding to Pale duplication is lethal in the diploid (BRIDGES and MORGAN 1923, LI 1927). In the triploid, however, it survives. The eye color of such a triploid (Pale deficiency/+/+) is practically normal; there is a slight dullness, but nothing comparable to the brown patches of the Plum-1/+/+ triploid. A simple deficiency of the brown locus does not exhibit the characteristics of Plum. It is possible, by making certain arbitrary assumptions with regard to genic balance, to consider Plum as a deficiency for a definite region having this effect. This is not in accord with our present knowledge of the behavior of deficiencies; but even this possibility can be excluded by procuring short deficiencies for the brown locus.

It is simpler at present to think of the change at the brown locus as a "mutation" of some sort. This is seen to be correlated with a change in chromosome structure. But the rearrangement involved another break in the chromosome. Does a similar situation exist in that case? Are "mutations" to be found in this region?

THE CHANGES AT THE LEFT BREAK OF THE INVERSION

It will be remembered that the left break of the Plum-2 inversion is between the locus of Bristle (54.7) and the spindle attachment. For this region a procedure similar to that adopted for the plexus-speck region was carried out. Not only were tests made with the mutants in the region, but there was also available a viable duplication. This is obtained from the II-Y translocation G, studied by RHOADES (1931), and includes the loci hooked (*hk*, 53.9), purple (*pr* 54.5), and light (*lt*, 55.0). Experiments were made with Plum-1 as well as with Plum-2.

Tests for allelism with the following genes in this region were carried out: hooked, purple, Bristle (recessive effects), light, rolled, thick, yellow bristle, and apterous. Of these, only light has its locus between Bristle and the spindle attachment—at the place of breakage in the Plum chromosome. Only light, an orange eye color, shows any signs of an allelic relation; the other tests were all negative, even as regards variegation.

The Plum-light compound is strikingly different from that of Plum with brown. In that case the variegation became almost imperceptible; but with light the mosaic character of the eye becomes more pronounced. On a light tan, almost yellow, ground color, extensive and distinct brown patches are spread. This difference from the brown compound made it necessary to examine the case more critically—a procedure called for in any event by its unusual, although predictable character.

Accordingly, heterozygotes for Plum-2 and a large number of different eye colors were observed. The types used were: carnation, cinnabar, claret, maroon, prune, purple, purploid, scarlet; combinations: purple cinnabar, scarlet claret, and vermilion garnet-2. Of all these, only the heterozygote of Plum-2 with vermilion garnet showed even a slight dilution. The relation of Plum to light remains unique.

There is one other specific relationship found among the eye colors of Drosophila, besides that of allelism. Certain eye colors, normally recessive, show visible effects in the heterozygous condition, when the fly is at the same time homozygous for other specific recessives (MORGAN and BRIDGES 1913, CREW and LAMY 1933, SCHULTZ unpublished). It was then necessary to determine the effect of heterozygous light in the presence of homozygous brown—which might be considered as equivalent to Plum. This was done, and served to exclude the possibility of such a conditioned dominance completely. The combination light brown/brown is practically normal brown; the effect of light upon Plum must therefore be regarded as a case of allelism.

It is then interesting to consider more closely the mosaic nature of the Plum/light eye. The ground color is very similar to the color of the light brown combination. This is a pale yellow, which in old age takes on a pinkish cast. The dilute areas in the Plum/light compound are somewhat darker, but this may be due simply to the effect of the neighboring brown areas. In the double compound Plum/light brown, the whole eye may sometimes look exactly like light brown, except for a few apricot-colored patches. The testis color of light is too close to that of Plum to be of value for the present problem.

In the Plum/light compound, the dilute areas may be interpreted as portions of the eye in which a mutation to light has taken place. This in combination with the dominant effect appears "light brown." The remaining dark areas would then be considered to show only the dominant effect of Plum. If this interpretation be accepted, then the Plum/light brown compound, in which the majority of the ommatidia have the color characteristic of the light brown combination, may indicate that the variegation for light and that for brown are correlated. However, the high frequency of the mutation to brown (Plum/brown is practically homogeneous)

makes it difficult to ascertain this, and special experiments must be devised to distinguish between a spurious association due to a high mutation frequency, and a real correlation. The important point at the present juncture is the indication that the light locus also undergoes variegation.

This can be shown in another way. The chief difficulty in the interpretation of the compounds of light with Plum arises from the dominant effect of Plum. But the dominant effect can be suppressed by the Pale duplication or in triploids, and the behavior of light can then be studied by itself. Therefore flies of the constitution Plum (1 or 2)/lt, Pale duplication were synthesized from crosses of Plum/Curly, Pale duplication/Stubble by light individuals. Instead of the wild-type color of the normal suppressed Plum fly, these showed a distinct purplish ground color, with darker purplish patches, resembling in their distribution those of the Plum/light compound. The variegation at the light locus appears to be a real phenomenon, not dependent on an interaction of a stable light mutation with the unstable brown locus. Results from observations of triploids of the constitution Plum/light/light confirm this. These show also a coarse mottling, and resemble the compounds, with the Pale duplication, just described.

The question again presents itself; can these patches result from a simple deficiency, for the locus of light? This appears unlikely, for several reasons. In the first place, a deficiency involving little more than the locus of light is already known (MORGAN, BRIDGES and SCHULTZ 1931) to have different properties than those observed in the case of Plum. Its compound with light is not as in the Plum/light Pale duplication flies, a purplish color, but closely resembles that of light itself. Moreover, the deficiency is an extreme Minute—which is not an insuperable difficulty, but deserves consideration. Secondly, unless it be postulated that the light locus does not produce its developmental effects autonomously, but is subject to the influence of neighboring tissues—for which there is no evidence at present—the purple, rather than wild-type color of the darker patches remains unaccounted for. Some phenomenon other than simple deficiency must be invoked to explain the results. They are, indeed, easily accounted for either as "mutation" in the orthodox sense or as due to the change brought about by the chromosome rearrangement—a position effect.

There remains the recessive lethal effect of Plum to be placed in its proper setting. It will be remembered that the presence of a duplication for the brown locus was of no avail. The homozygotes for Plum-2 die in very late pupal stages, just before hatching, both in the presence of the duplication and without it. Their eye color and general morphology can be made out on dissection, however, and in certain stocks of Dr. BRIDGES, occasional homozygotes even survive, affording a check of the dissections. Individuals containing Plum-2 in homozygous condition have a pale yellow

eye color, with occasional apricot patches. This is exactly as would be expected from the behavior of Plum-2 with light and brown. The homozygote should resemble, as it does, the compound Plum-2/light brown. In other respects these individuals (even the survivors) are decidedly abnormal. The wings are somewhat broad at the base and flimsy. In a number of cases dissected out of the puparium, patches devoid of ommatidia were found in the eye, and weakly chitinized, blackened places at the joints of the legs.

Does this recessive lethal effect bear any relation to the left break of the inversion? If it does, not one but two "mutations"—possibly on either side of the break—will have been shown to lie here. By the use of the duplication previously mentioned for the loci hooked purple and light, it was in fact possible to obtain viable homozygotes for Plum-2, and establish a stock thereof.

Duplication G is attached to a Y chromosome. Accordingly, males were prepared which carried the Y chromosome with duplication G, and in their second chromosomes Bristle Plum-2 cinnabar/Curly. These were mated to Plum-2/Curly females. The results were:

Plum-2	Curly	*Bl Pm*^{2}	*Cy cn*	*Bl Pm*^{2}
♀♀	♂♂	♀♀	♂♂	♂♂
32	35	54	25	33

The last named class is homozygous Plum-2 since it is not Curly. The appearance of this class only in the males means that duplication G is the responsible factor in their survival. The locus of the recessive lethal effect in Plum-2 is at the left break of the inversion.

Comparison of these homozygotes with those which occasionally survive without the duplication is interesting. They are practically, normal in viability, except for the slight decrease due to the duplication and the homozygous Plum-2 duplication G stock is easily kept. Their eyes are brown in color, similar to the Plum/brown compound; the effect of light is completely suppressed by the duplication. A similar phenomenon is observed in flies of the composition Plum/light brown, duplication G. These also are clear brown in young flies, with some mottling as age advances. As regards the brown locus, each Plum chromosome behaves to the other as if a mutation to brown were present. This extends to the testis sheath, where the variegation found in the ordinary Plum/brown compound is clearly manifested. The mosaic character is apparently not dependent on heterozygosity for the locus concerned. This was already evident from the presence of patches in the eyes of the homozygote without the duplication. But although the mosaic character persists, none of the abnormalities of the ordinary homozygote appear in the presence of the duplication.

It is clear from the foregoing that at both breaks a complex of changes

has occurred, which cannot readily be interpreted as simple deficiency phenomena. They can be interpreted as resulting from the chromosome rearrangement which is associated with them. An interesting possibility is now raised by the position effect hypothesis. Six alleles of brown have been studied by BRIDGES, one of them possibly a deficiency. None of these shows the dominant effects of the Plum alleles. Are these dominant effects due to a cooperative action of the genes involved in the rearrangement?

THE DOMINANT EFFECT OF PLUM

The obvious method for the analysis of a dominant effect is to consider the mode of its suppression. The data with Pale duplication already have shown that Plum is suppressed by the addition of an extra wild-type allele of brown. It remained possible that a suppression could also be effected by additional allele of light. In that case, the dominant effect would have been due to the interaction of the two loci, neither of which by itself could produce the change.

The data already given for the effect of duplication G on Plum-2 disprove this. All Plum-2 males in that experiment carried the duplication, and were normal Plum-2. A similar experiment was performed with Plum-1. Aristaless dachs Bristle curved speck/dumpy black Plum-1 females were crossed to Curly/translocation G males. In these crosses the translocation appears only in the males, since deficiency G is lethal in heterozygous condition. The Curly males are the class of interest, since these carry the duplication. The results of the cross were:

dp b Cy Pm		*Cy Bl*		*Bl*		*Pm*		Crossovers (mostly minutes)
♀	♂	♀	♂	♀	♂	♀	♂	
89	66	128	49	1	101	0	90	27

There was no suppression of Plum-1 by duplication G, as there was no suppression of Plum-2. It would seem therefore that the dominant effect is concerned solely with the brown locus.

During the course of the work with duplication G, however, it was found that suppression of Plum-1 and Plum-2 could occur even when no additional allele of brown was present. It was found on analysis that the suppression is due to the presence of an extra Y chromosome, an effect similar to that reported by GOWEN and GAY (1933). Not only the dominant eye color, but the instability for minus and light as well are suppressed. And the suppression has been found, by experiments with fragments of the Y, in the presence of only the short arm of this chromosome. These results will be reported separately. They concern us here only in so far as they make it necessary to examine the suppression of Plum by the brown locus more critically.

This is made all the more necessary by a certain parallelism between the effects of Pale duplication and the Y chromosome on the homozygote which contains duplication G. To obtain the former, Plum-2/Curly, Stubble $C_{IIIR\ MO}$/Pale duplication were crossed to homozygous Plum-2 males carrying duplication G. The results were:

Cy Pm Sb		*Cy*		*Cy Pm*		*Pm Sb*		+		*Pm·Sb*
♀♀	♂♂	♀♀	♂♂	♀	♂	♀	♂	♀	♂	$\overline{Pm}$ ♀
48	48	56	39	1	0	1	56	0	44	2

The not-Curly classes are of interest. The two females did not contain the duplication, and were the extreme homozygous type already described. The "*Pm Sb*" class was brown-eyed, typical homozygous Plum-2 with duplication G. The wild-type males carry in addition the Pale duplication, whose effect on the homozygote is precisely the same as its effect on the heterozygote. It may be remarked, that if Plum could still be considered a simple deficiency for brown, this result would completely exclude the possibility. For the addition of a duplication to the homozygote restores the ratio of alleles characteristic of the heterozygote, and these flies should be Plum. They are, however, as completely suppressed Plum as is the heterozygote. This in itself is not disturbing, as far as the mutation or position effect interpretation is concerned, and can easily be accounted for on these hypotheses.

But homozygous Plum females containing duplication G are also suppressed Plum; this is a constant feature of the stock of homozygous Plum with duplication G. It is, of course, due to the additional Y introduced into the female with the duplication. The suppression is not so complete as that effected by the Pale duplication, but nevertheless makes additional experiments necessary.

The suppression of Plum in single dose in triploids made it possible to pursue the problem further. If the brown locus is really concerned with the dominance, its removal by a deficiency will cause the reappearance of the dominant character, other conditions remaining constant. The material for such an experiment is furnished by the Pale deficiency discussed previously, which is viable in triploids as a fly with somewhat narrowed wings and slight body build. Accordingly, triploids were obtained which contained Plum-1 in single dose. Some already contained Stubble, disregarded in the counts, except for the Pale deficiency flies, which necessarily contain it. These were then crossed to males of the constitution Pale deficiency/Plexate, Pale duplication/Stubble $C_{IIIR\ MO}$. The Plexate character is completely suppressed in triploids, so that in the progeny of this cross, there are only wild-type and Pale deficiency triploids to be distinguished in re-

gard to their behavior with Plum. The disjunction of Plum-1 in the triploid is not random; most of the diploid gametes from a Plum-1 triploid contain the Plum-1 chromosome. Individuals of the desired constitution—Plum-1/Pale deficiency/+ were thus easily obtained. The results of the cross were:

	Intersexes	3*N*		Pale def.	3*N*	
2*N*	+	Supp. *Pm*[1]	+?	*Pm Sb*	*Sb*	Super♂
210	64	32	25	15	3	5

The distinction between wild-type and Plum in triploids is not easily made. Probably most of the apparent wild-type triploids are really Plum, with the additional suppression of a Pale duplication. But the triploids carrying Pale deficiency and Plum-1 manifest Plum-1 to even a greater extent than does the diploid heterozygote. The ground color is somewhat lighter and the patches more frequent. The Pale deficiency triploids without Plum in this cross as in many others were completely normal in eye color. The loss of the extra brown locus restores the dominant effect of Plum-1. This result is consistent with the assumption that brown is the locus concerned in the dominant effect. It finds corroborative support in certain observations of triploids of the constitution Plum/brown/+. These, instead of the normal suppression of Plum in the triploid, approach the diploid heterozygote in appearance. Further data of this type are desirable, however, although the result already seems clear. The presence of a mutant brown allele reinforces the action of Plum. All in all, the evidence favors the view that the dominant effect in Plum is a specific property of the brown locus.

This is further borne out by the negative results of the experiments with deficiency G for the light locus, which also survives in triploids. Triploid females containing Plum-1 in single dose were crossed to Curly/translocation G males. All not-Curly individuals which receive no Y from the father carry the deficiency. The results were:

2*N*	3*N*				Inter-	
	supp. *Pm*	Supp. *Pm Cy*	*Cy*+?	+?	sexes	Super♂
135	4	5	7	2	69	3

The not-Curly triploids therefore carry the deficiency, which survives and is almost normal except for bristle length and wing shape. The absence of the light locus produces no effect on the manifestation of Plum. Combined with the failure of the duplication to suppress Plum, these data reinforce the conclusion that the dominant effect of Plum is a function not of the light, but of the brown locus.

DOMINANT EYE COLORS AND THE POSITION EFFECT HYPOTHESIS

The essential requirement of the position effect hypothesis is the regular occurrence of a definite "mutation" with a definite chromosome break. The mutation may involve both, or only one of the genes newly juxtaposed at the place of rearrangement. The important point is the correlated appearance of the mutation and the chromosome fracture at a given locus.

The alternative hypothesis, the frequent occurrence of gene mutations in the ordinary sense at points of breakage, is really one form of the position effect hypothesis. It involves the assumption that the breakage of the chromosome *per se* is responsible for an upset in gene structure. On closer inspection, this means the separation of a gene from its normal neighbors, and implies a relationship of some sort between them.

The other alternative is a loss of material at the point of breakage. This can readily be excluded by the application of the usual tests for deficiency (BRIDGES, MOHR), in those cases where known genes are involved in the mutation.

For the dominant eye colors which are the subject of this paper, the data are consistent with the position effect hypothesis. The work of VAN ATTA, and particularly of GLASS, has shown that in every case, the dominant effect is correlated with a break in a specific region. To their work we have added in several cases the correlated occurrence of a mutation with the other break in the chromosome. And every test for deficiency imposed upon the mutations at both breaks has yielded negative results.

The analysis further indicated no correlative action of the two genes in the production of the dominant effect. This, taken together with VAN ATTA'S and GLASS'S cases in which a similar mutation is found at the brown locus, with a different kind of translocation, would seem to indicate that the separation of brown from its normal neighbors is sufficient to accomplish the mutation.

As regards the light locus, there are, however, data on two translocations in which a breakage has taken place close to light (translocation G, RHOADES 1931 and translocation H, SCHULTZ unpublished) in which there is no mutation at the light locus. These cases, if the break be considered as close enough to light, which makes some caution necessary, provide evidence of an important kind for the position effect hypothesis. If it can be proved that only certain rearrangements give specific effects, the hypothesis becomes established. If a mutation at the light locus occurs only as the result of its juxtaposition to brown or its neighbors, and not when other rearrangements occur, the proof for this case might be regarded as complete. We do not at the present know precisely the relations of brown and light in these inversions; the possibilities are shown in figure 7, and it

should be a simple matter to distinguish between them. If the two eye color genes are really adjacent, a variety of speculations become of interest.

Whether the lethal effect is at the same locus as the eye color mutation touches another question of interest. It is possible that not only the genes adjacent to the breakage, but also those farther removed may be affected. A series of data on changes of dominance of "normal genes" in duplicating sections of chromosome (DOBZHANSKY and STURTEVANT 1932) is suggestive in this respect. The alternative in these cases is an upset of genic balance; but even this must be an ordered change, since the closer the break, the greater the change from the normal dominance effect.

SUMMARY

1. A new dominant eye color in *Drosophila melanogaster*, called Plum-2 is a member of the series of alleles of brown (Plum or Dilute) studied by VAN ATTA and GLASS.

2. The Plum-2 chromosome contains an inversion, cytologically demonstrated, whose ends are respectively near the loci of light and of brown (figure 7). The Plum-2 mutation is inseparable from the ends of the inversion.

3. Plum-2 behaves as an allele to both mutants light and brown. It exhibits instability for these loci, and also for minus (close to brown). Plum-1 exhibits a behavior similar to Plum-2.

4. A series of experiments, designed to test the hypothesis that the mutations at the breaks are due to simple deficiencies, yielded consistently negative results.

5. The dominant effect in Plum-1 and Plum-2 is a function of the brown locus only.

6. The data presented are consistent with the hypothesis that the behavior of genes is a function of their position in the chromosome, that is, depends upon their relation to other genes.

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